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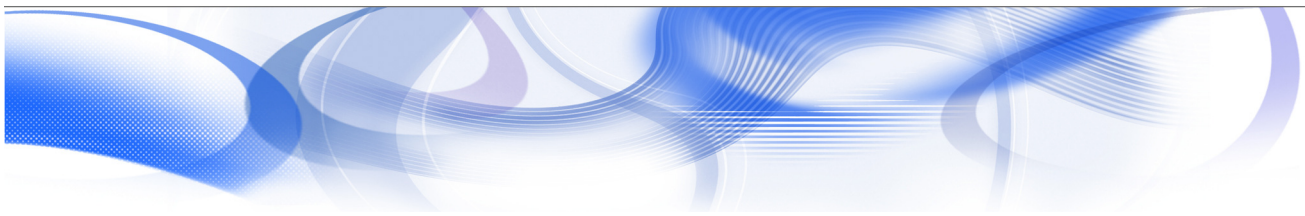

# Possible Application of Non-Testing Methods in Setting Environmental Quality Standards (EQS)

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EUR 23758 EN - 2008

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http:// http://ecb.jrc.europa.eu/QSAR
http://ec.europa.eu/dgs/jrc/



JRC 50067

EUR 23758 EN

ISSN 1018-5593

Luxembourg: Office for Official Publications of the European Communities



*Printed in Italy*

# ABSTRACT

The Water Framework Directive is one of the most important pieces of European environmental legislation in recent years, requiring all inland and coastal waters to achieve ‘‘good status’’ by 2015. Article 16 of the Directive describes how and by when Environmental Quality Standards (EQS) for pollutants should be developed, and states that pollutants presenting a significant risk to or via water should be identified by the European Commission and classified as priority substances, with the most hazardous of these classified as priority hazardous substances.

Generation of ecotoxicity test data is one option for filling gaps when deriving EQS but there are also options that avoid testing, such as the use of (Quantitative) Structure Activity Relationships ([Q]SARs), Quantitative Structure-Property Relationships (QSPRs), Activity-Activity Relationships (AARs), Quantitative Structure Activity-Activity Relationships, or read-across from similar substances. All of these non-testing methods are based on the idea that properties (including biological activities) of a chemical substance depend on its intrinsic nature and can be directly predicted from its molecular structure and inferred from the properties of similar compounds whose activities are known.

This report explores application of the widely used and freely available EPIWIN suite of QSARs, particularly the ecotoxicity software ECOSAR, for derivation of EQS under the WFD. The predictive ability of these QSARs is examined using the 33 priority substances in the Water Framework Directive Daughter Directive on Priority Substances, plus the additional substances recommended for inclusion by the European Parliament, as representative substances for EQS derivation. We also investigated use of the OECD QSAR Application Toolbox version 1 for read across of data. We address the following questions:

1. How effective are the QSARs in EPIWIN at identifying the selected substances as Persistent, Bioaccumulative and Toxic (PBT) or very Persistent and very Bioaccumulative (vPvB)? These substances would potentially be classifiable as priority hazardous substances and would also require consideration of sediment toxicity and secondary poisoning through the food chain.
2. How accurate and precise is ECOSAR at estimating acute and chronic toxicity for fish, invertebrates and algae for these substances?
3. How similar are EQS for these substances based on measured data and ECOSAR estimates?
4. To what extent is the most sensitive trophic group accurately predicted by ECOSAR for acute or chronic exposure?
5. Can a formal approach for defining similarity for read-across help to group substances in a defensible way that increases the amount of data available overall for EQS derivation?

The following conclusions and recommendations apply generally on the assumption that the substances analysed in this report are representative of the types of substances for which EQS may also need to be set in the future.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Annual average concentration (arithmetic mean) |
| AAR | Activity-Activity Relationships |
| AF | Assessment Factor |
| BCF | Bioconcentration Factor |
| ChV | Chronic Toxicity Value |
| EQS | Environmental Quality Standards |
| MAC | Maximum Allowable Concentration |
| MoA | Mode of Action |
| NOEC | No Observed Effect Concentration |
| OECD | Organisation for Economic Co-operation and Development |
| PAH | Polyaromatic hydrocarbons |
| PBT | Persistent, Bioaccumulative and Toxic substance |
| PNEC | Predicted No Effect Concentration |
| Kow | Octanol-water partition coefficient |
| QAAR | Quantitative Structure Activity-Activity Relationship |
| QSAR | Quantitative Structure-Activity Relationship |
| QSPR | Quantitative Structure-Property Relationship |
| REACH | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| TGD | European Technical Guidance Document on Risk Assessment |
| vPvB | very Persistent and very Bioaccumulative |
| WFD | Water Framework Directive |

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# 1. Introduction

The Water Framework Directive (WFD; EC 2000) is one of the most important pieces of European environmental legislation in recent years, requiring all inland and coastal waters to achieve ‘‘good status’’ by 2015. It will do this by establishing a river basin district structure within which demanding environmental objectives will be set, including ecological targets for surface waters and the use of environmental quality standards (EQS) for individual chemical pollutants. Article 16 of the Directive describes broadly how and by when EQS for pollutants should be developed, and states that pollutants presenting a significant risk to or via water should be identified by the European Commission (EC) and classified as priority substances, with the most hazardous of these classified as priority hazardous substances. All of these substances will become Annex X of the WFD after adoption by the European Parliament and the Council. For priority substances and priority hazardous substances, measures should aim at progressive reduction and cessation of discharges, respectively, by 2025. The approach used to derive EQS for priority substances was developed by the Fraunhofer Institute (FHI) under contract to the EC using guidelines published by Lepper (2005). This approach was based largely on current European Technical Guidance Document (TGD) methods for derivation of predicted no effect concentrations (PNECs) in chemical risk assessment (EC 2003).

On 17 July 2006, the EC released the proposed Daughter Directive to the WFD to deal with the control of priority substances. The Daughter Directive begins with a statement of its main aim and the general context that has led to the requirement for such a directive. The aim is to ‘‘ensure a high level of protection against risks to or via the aquatic environment stemming from . . . 33 priority substances and certain other pollutants by setting environmental quality standards (EQS)’’ (EC 2006). In the Daughter Directive, annual average EQS are set for surface waters for all 33 priority substances. Table 1 provides a summary of the proposed standards. No sediment standards are included, but member states are required under Article 2(3) to ensure that biota concentrations do not exceed 20 μg $kg^{-1}$ methylmercury, 10 μg $kg^{-1}$ hexachlorobenzene, and 55 μg $kg^{-1}$ hexachlorobutadiene. They can do this either by direct measurement of concentrations in biota or by extrapolating back to water concentrations that might lead to biota concentration limits under equilibrium partitioning conditions.

There has been a recent debate between the EC, the Council of Ministers and the European Parliament about whether further substances should be added to the Daughter Directive. Investigation of the substances proposed for inclusion by the European Parliament (Table 2) shows that acute or chronic ecotoxicity data are currently unavailable for some or all of the trophic levels required by the EQS derivation methodology described by Lepper (2005). The consequence of this is that either no EQS can be set, or one must be set using very large assessment factors (AFs), which makes the resulting standard highly conservative. It is also likely that during regular reviews mandated by the WFD further substances with few or no ecotoxicity data will be prioritized by the EC for derivation of EQS.

Generation of ecotoxicity test data is one option for filling gaps when deriving EQS but there are also options that avoid testing, such as the use of (Quantitative) Structure Activity Relationships ([Q]SARs), Quantitative Structure-Property Relationships (QSPRs), Activity-Activity Relationships (AARs), Quantitative Structure Activity-Activity Relationships, or read-across from similar substances. All of these non-testing methods are based on the idea that properties (including biological activities) of a chemical substance depend on its intrinsic nature and can be directly predicted from its molecular structure and inferred from the properties of similar compounds whose activities are known (Bassan and Worth 2008). In its only reference to QSAR approaches the WFD EQS guidance document (Lepper 2005) states that *“Quantitative structure activity relationship (QSAR) estimates for toxicity may be*

*referred to as supporting information in the derivation of QS, but such QSAR estimates cannot exclusively be used to derive a standard (i.e., experimental toxicity data supporting the QSAR estimates are required).*" One of the ways in which Lepper and his colleagues used QSARs in support of EQS derivation was when the absence of a single piece of test data for an organism not expected to be sensitive to a substance resulted in application of a large AF. Review of the 33 WFD data sheets prepared in support of the Daughter Directive shows that AFs to derive annual average EQS were >10 for only three substances, and in these cases the increase was to an AF of 50. The AF was increased for dichloromethane because of a lack of reliable invertebrate data. The AF was increased for naphthalene and octylphenol because of a lack of reliable algal data. There were no cases amongst the datasheets when the AF used to calculate a Maximum Allowable Concentration EQS was greater than the minimum of 100. However, there may be other ways in which QSARs could be of use in setting EQS, which would help in minimizing costs and animal experimentation.

This report explores application of the widely used (OECD 2007a) and freely available EPIWIN suite of QSARs, particularly the ecotoxicity software ECOSAR, for derivation of EQS under the WFD. The predictive ability of these QSARs is examined using the 33 priority substances in the Daughter Directive, plus the additional substances recommended for inclusion by the European Parliament, as representative substances for EQS derivation. We also investigated use of the recently released OECD QSAR Application Toolbox version 1[1] for read across of data. We address the following questions:

1. How effective are the QSARs in EPIWIN at identifying the selected substances as Persistent, Bioaccumulative and Toxic (PBT) or very Persistent and very Bioaccumulative (vPvB)? These substances would potentially be classifiable as priority hazardous substances and would also require consideration of sediment toxicity and secondary poisoning through the food chain.

2. How accurate and precise is ECOSAR at estimating acute and chronic toxicity for fish, invertebrates and algae for these substances?

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5. Can a formal approach for defining similarity for read-across help to group substances in a defensible way that increases the amount of data available overall for EQS derivation?

[1] http://www.oecd.org/document/23/0,3343,en_2649_34377_33957015_1_1_1_1,00.html#Download_QSAR_AT

**Table 1** **Proposed Water Framework Directive Environmental Quality Standards (EQS) for Annex X Priority Substances (* = Priority Hazardous Substance). AA = Annual average (arithmetic mean), MAC = Maximum Allowable Concentration. All values are in µg $l^{-1}$ total concentration, except for metals, which are dissolved concentrations (EC 2006).**

| No. | Name of substance | CAS # | AA-EQS Inland surface waters | AA-EQS Other surface waters | MAC-EQS Inland surface waters | MAC-EQS Other surface waters | Changes from earlier drafts of Directive |
|---|---|---|---|---|---|---|---|
| 1 | Alachlor | 15972-60-8 | 0.3 | 0.3 | 0.7 | 0.7 | |
| 2* | Anthracene | 120-12-7 | 0.1 | 0.1 | 0.4 | 0.4 | |
| 3 | Atrazine | 1912-24-9 | 0.6 | 0.6 | 2 | 2 | No longer classed as a Priority Hazardous Substance |
| 4 | Benzene | 71-43-2 | 10 | 8 | 50 | 50 | |
| 5* | Pentabromodiphenylether | 32534-81-9 | 0.0005 | 0.0002 | not applicable | not applicable | MACs no longer proposed (these were 0.006 µg $l^{-1}$ for inland waters and 0.002 µg $l^{-1}$ for other surface waters) |
| 6* | Cadmium and its compounds (depending on water hardness classes) | 7440-43-9 | ≤ 0.08<br>0.08<br>0.09<br>0.15<br>0.25 | 0.2 | ≤ 0.45 (@ <40 mg $CaCO_3$ $l^{-1}$)<br>0.45 (@ 40-<50 mg $CaCO_3$ $l^{-1}$)<br>0.6 (@ 50-<100 mg $CaCO_3$ $l^{-1}$)<br>0.9 (@ 100 - <200 mg $CaCO_3$ $l^{-1}$)<br>1.5 (@ ≥200 mg $CaCO_3$ $l^{-1}$) | | The original band of 40-<100 mg $CaCO_3$ $l^{-1}$ has been split into two bands: 40-<50 and 50 - <100 mg $CaCO_3$ $l^{-1}$ |
| 7* | $C_{10-13}$ Chloroalkanes | 85535-84-8 | 0.4 | 0.4 | 1.4 | 1.4 | |
| 8 | Chlorfenvinphos | 470-90-6 | 0.1 | 0.1 | 0.3 | 0.3 | |
| 9 | Chlorpyrifos | 2921-88-2 | 0.03 | 0.03 | 0.1 | 0.1 | |
| 10 | 1,2-Dichloroethane | 107-06-2 | 10 | 10 | not applicable | not applicable | MACs no longer proposed (these were 120 µg $l^{-1}$ for all waters) |
| 11 | Dichloromethane | 75-09-2 | 20 | 20 | not applicable | not applicable | MACs no longer proposed (these were 240 µg $l^{-1}$ for all waters) |
| 12 | Di(2-ethylhexyl)phthalate (DEHP) | 117-81-7 | 1.3 | 1.3 | not applicable | not applicable | |
| 13 | Diuron | 330-54-1 | 0.2 | 0.2 | 1.8 | 1.8 | |
| 14* | Endosulfan | 115-29-7 | 0.005 | 0.0005 | 0.01 | 0.004 | |
| 15 | Fluoranthene | 206-44-0 | 0.1 | 0.1 | 1 | 1 | |
| 16* | Hexachlorobenzene | 118-74-1 | 0.01 | 0.01 | 0.05 | 0.05 | All values are now less stringent. The AA in earlier drafts was 0.0002 µg $l^{-1}$ |

| No. | Name of substance | CAS # | AA-EQS Inland surface waters | AA-EQS Other surface waters | MAC-EQS Inland surface waters | MAC-EQS Other surface waters | Changes from earlier drafts of Directive |
|---|---|---|---|---|---|---|---|
| | | | | | | | and the MAC was 0.002 µg $l^{-1}$. |
| 17* | Hexachlorobutadiene | 87-68-3 | 0.1 | 0.1 | 0.6 | 0.6 | All values are now less stringent. The AA in earlier drafts was 0.003 µg $l^{-1}$ and the MAC was 0.04 µg $l^{-1}$. |
| 18* | Hexachlorocyclohexane | 608-73-1 | 0.02 | 0.002 | 0.04 | 0.02 | |
| 19 | Isoproturon | 34123-59-6 | 0.3 | 0.3 | 1 | 1 | |
| 20 | Lead and its compounds | 7439-92-1 | 7.2 | 7.2 | not applicable | not applicable | The AA is now less stringent (it was 2.1 µg $l^{-1}$ in earlier drafts). MACs no longer proposed (these were 2.8 µg $l^{-1}$ for all waters) |
| 21* | Mercury and its compounds | 7439-97-6 | 0.05 | 0.05 | 0.07 | 0.07 | |
| 22 | Napthalene | 91-20-3 | 2.4 | 1.2 | not applicable | not applicable | MACs no longer proposed (these were 28.8 µg $l^{-1}$ for inland waters and 14.4 µg $l^{-1}$ for other surface waters) |
| 23 | Nickel and its compounds | 7440-02-0 | 20 | 20 | not applicable | not applicable | The AA is now less stringent (it was 3.8 µg $l^{-1}$ in earlier drafts). MACs no longer proposed (these were 13.6 µg $l^{-1}$ for all waters) |
| 24* | Nonylphenol | 25154-52-3 | 0.3 | 0.3 | 2 | 2 | |
| 25 | Octylphenol | 1806-26-4 | 0.1 | 0.01 | not applicable | not applicable | AA values have now been rounded down (they were 0.12 and 0.012 µg $l^{-1}$ respectively). MACs no longer proposed (these were 0.13 µg $l^{-1}$ for all waters) |
| 26* | Pentachlorobenzene | 608-93-5 | 0.007 | 0.0007 | not applicable | not applicable | MACs no longer proposed (these were 0.08 µg $l^{-1}$ for inland waters and 0.008 µg $l^{-1}$ for other surface waters) |
| 27 | Pentachlorophenol | 87-86-5 | 0.4 | 0.4 | 1 | 1 | |
| 28* | Polyaromatic hydrocarbons (PAH) | | | | | | |
| | *Benzo(a)pyrene* | 50-32-8 | 0.05 | 0.05 | 0.1 | 0.1 | |
| | *Benzo(b)fluoranthene* | 205-99-2 | Σ=0.03 | Σ=0.03 | not applicable | not applicable | |
| | *Benzo(k)fluoranthene* | 191-24-2 | | | | | |

| No. | Name of substance | CAS # | AA-EQS Inland surface waters | AA-EQS Other surface waters | MAC-EQS Inland surface waters | MAC-EQS Other surface waters | Changes from earlier drafts of Directive |
|---|---|---|---|---|---|---|---|
| | *Benzo(g,h,i)perylene* | 207-08-9 | Σ=0.002 | Σ=0.002 | not applicable | not applicable | |
| | *Indeno(1,2,3-cd)pyrene* | 193-39-5 | | | | | |
| 29 | Simazine | 122-34-9 | 1 | 1 | 4 | 4 | No longer classed as a Priority Hazardous Substance |
| 30* | Tributyltin compounds | 688-73-3 | 0.0002 | 0.0002 | 0.0015 | 0.0015 | |
| 31 | Trichlorobenzenes (all isomers) | 12002-48-1 | 0.4 | 0.4 | not applicable | not applicable | No longer classed as a Priority Hazardous Substance |
| 32 | Trichloromethane | 67-66-3 | 2.5 | 2.5 | not applicable | not applicable | MACs no longer proposed (these were 30 $\mu g\ l^{-1}$ for all waters) |
| 33 | Trifluralin | 1582-09-8 | 0.03 | 0.03 | not applicable | not applicable | MACs no longer proposed (these were 0.4 $\mu g\ l^{-1}$ for all waters) |

**Table 2 Additional WFD Priority Substances proposed by the European Parliament**

| **Substance** | **Cas #** |
|---|---|
| Perfluorooctane sulphonic acid | 1763-23-1 |
| *Potassium salt* | 2795-39-3 |
| *Ammonium salt* | 29081-56-9 |
| Perfluorooctanoic acid (PFOA) | 335-67-1 |
| *Ammonium perfluorooctanoate* | 3825-26-1 |
| Amidotrizoate | 131-49-7 |
| AMPA (glyphosate metabolite) | 1066-51-9 |
| Bentazon | 25057-89-0 |
| Bisphenol A | 80-05-7 |
| 4 4'-biphenol | 92-88-6 |
| Carbamazepine | 298-46-4 |
| Clotrimazole | 23593-75-1 |
| Dibutylpthalate | 84-74-2 |
| Diclofenac | 15307-86-5 |
| Dicofol | 115-32-2 |
| DTPA | 67-43-6 |
| EDTA | 60-00-4 |
| ETBE | 637-92-3 |
| Glyphosate | 1071-83-6 |
| HHCB | 1222-05-5 |
| Iopamidol | 60166-93-0 |
| Mecoprop | 7085-19-0 |
| 4-methylbenzylidene camphor | 36861-47-9 |
| Musk ketone | 81-14-1 |
| Musk xylene | 81-15-2 |
| MTBE | 1634-04-4 |
| Napthalene-1,5-disulfonate | 81-04-9 |
| Octylmethoxycinnamate | 5466-77-3 |
| Quinoxyfen (5,7-dichloro-4-(p-fluorophenoxy)quinoline | 124495-18-7 |
| Tetrabromobisphenol A (TBBP-A) | 79-94-7 |
| Tonalid (AHTN) | 21145-77-7 |

# 2. Methods

## 2.1 Available computational methods for deriving EQS

There have been several recent reviews of available QSARs and QSPRs (referred to collectively as QSARs from now on in this report) for estimating persistence, bioaccumulation and aquatic toxicity, which are the most relevant endpoints for EQS derivation. Under REACH it is possible to use data from QSARs instead of experimental data if four conditions are fulfilled (Bassan and Worth 2008):

1. The model used is shown to be scientifically valid.

2. The model used is applicable to the chemical of interest.

3. The prediction is relevant for the regulatory purpose.

4. Appropriate documentation on the method and result is given (e.g., by using the QSAR Model Reporting Format recommended by the European Commission[2]).

It seems reasonable to expect the same standards applied to the acceptability of QSARs under the REACH regulations to apply equally to their use when deriving EQS under the Water Framework Directive.

*Estimation of persistence*

Pavan and Worth (2006, 2008) review the estimation models for biodegradation used in many jurisdictions to assess whether a substance is likely to persist in the environment. They point out that under the WFD persistency in the environment is an important criterion for the prioritisation of chemicals as dangerous to the aquatic environment. Most current biodegradability QSARs, such as BIOWIN, use an approach in which a substance is decomposed into several fragments and the model expresses biodegradability as a function of the contribution of each fragment in the molecule. These types of models are successful in predicting ready biodegradability for between 72-80% of substances and non-ready biodegradability for between 80-85% of substances. BIOWIN v4.02 provides a battery of models that give a qualitative yes/no prediction for ready biodegradability. The USEPA's PBT profiler[3] takes the results from the BIOWIN 3 module and converts these to DT50 estimates in days, which can then be used for comparison with regulatory criteria for persistence. In contrast, the EU TGD and REACH guidance suggest that the outputs from BIOWIN 1 and 2 should be used conservatively to confirm that a substance is not readily biodegradable, with outputs predicting fast biodegradation not taken into account. In support of this, Pavan and Worth (2006) also suggest that BIOWIN 5 and 6 can be used more reliably to predict that a substance is *not* degradable. However, since the P criterion in PBT is expressed in days, qualitative yes/no outputs will need to be converted into quantitative outputs in a way similar to the PBT profiler.

[2] http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=QRF
[3] www.pbtprofiler.net

*Estimation of bioaccumulation*

Pavan et al. (2006, 2008) review QSAR models for bioconcentration and describe how initial attempts to model log BCF by log Kow alone were unsatisfactory. The BCFWIN software (Meylan et al. 1999) in EPIWIN is based on measured BCF data for 694 chemicals, and includes different models for different log Kow ranges, and correction factors for certain functional groups. However, it tends to underestimate bioaccumulation potential. Dimitrov et al. (2003) proposed a model for evaluating maximum bioconcentration potential (log $BCF_{MAX}$) which identifies all chemicals with high bioconcentration potential, but produces a large number of false positives. Dimitrov et al. (2005) introduced a further model based on the log $BCF_{MAX}$ approach – the BCF base-line – which allows reduction of bioconcentration potential by use of mitigating properties such as molecular size, flexibility, ionisation and biotransformation. This approach to modelling BCF has been incorporated into the CATABOL software.

Some approaches to estimating BCF, such as solvatochromic descriptor and molecular connectivity index and fragment constant approaches do not require log Kow as an input parameter. However, despite advances in the use of alternative parameters, log Kow remains a key parameter in many estimates of bioaccumulation. Dearden and Worth (2007) provide clear, step-by-step guidance on how to select software and QSPRs for estimating physicochemical properties such as log Kow. They suggest that QSPR estimates for such parameters should be obtained from at least two different software packages or methods, including use of measured values if these are available. However, they caution against unquestioning preference of measured over estimated values. This is because the applicability domain of property prediction software uses training sets of many thousands of diverse chemicals so it is wide, and experimentally-derived values are also subject to error (e.g., a mean error on log Kow of ~0.3 log unit). Dearden et al. (2003) compared estimates of log Kow by 14 software programs for a 138-chemical test set. They found that for the top six software packages 88.4 - 94.2% of predicted values were within $\pm$ 0.5 log units of measured values (EPIWIN's KOWWIN=89.1%) and the standard error ranged from 0.27 - 0.34 log unit (KOWWIN=0.34). Sakuratani et al. (2007) also found that for a test set of 134 simple organic chemicals KOWWIN predicted log Kow for the majority (130) reasonably well and with a smaller standard error than several other programs.

Other promising approaches for estimating B in PBT include partial order ranking (Walker and Carlsen 2002, Carlsen and Walker 2003) and structurally-based PBT screening (Papa and Gramaticus 2006).

In risk assessment and EQS derivation the identification of bioaccumulation in potential food items, such as fish, triggers further investigation of possible secondary poisoning up the food chain from contaminated fish to mammalian and avian predators. This is why biota-based EQS are proposed by the EC for hexachlorobenzene, hexachorobutadiene and methyl mercury. It would be very useful in EQS derivation if QSARs for mammalian toxicity were available that could translate estimates of priority substance concentration in fish into estimates of toxicity in predatory mammals. Unfortunately, however, when compared with aquatic toxicity QSARs (see later) there is only a small number of published mammalian toxicity QSARs and their accuracy is questionable (Tsakovska et al. 2008). It is therefore not currently possible to use QSARs to estimate secondary poisoning with sufficient reliability to help in the derivation of EQS.

*Estimation of aquatic toxicity*

Netzeva et al. (2007, 2008) review the use of QSARs for estimating aquatic toxicity. These are generally based on functional classes, mode or mechanism of action, or statistical analyses of

descriptor properties for fish, invertebrates or algae. ECOSAR, implemented in EPIWIN, uses several class-specific log Kow-based QSARs based on measured data to provide estimates for fish, invertebrates and algae. Several other QSAR software applications are available for estimating toxicity and there is a very extensive literature on QSARs for specific combinations of chemical classes, modes of action and organisms.

However, the utility and predictive power of currently available models can be rather low. For example, de Roode et al. (2006) examined ECOSAR, TOPKAT and two QSARs for polar and non-polar narcosis. They found that the QSARs were unable to provide estimates for 28% of the test set they used. Correlations between measured and estimated effects were significant for TOPKAT and the QSAR for polar narcosis, but poor for ECOSAR and the non-polar narcosis QSAR. When the authors allowed up to a 5-fold difference between estimated and measured values, "correct" predictions occurred for 77%, 54%, 68% and 91% respectively for ECOSAR, TOPKAT, and the polar and non-polar QSARs. Netzeva and Worth (2007) found a good correlation ($r^2$=0.81) between acute fish toxicity estimates by ECOSAR and TOPKAT for 341 pthalates, but the limited availability of measured data for these substances precluded comparisons of estimated and measured values.

Netzeva et al. (2007) concluded from their extensive review that narcosis is the best represented MoA for QSAR models, and that more and better quality QSAR models for estimating aquatic toxicity are available in the order fish > daphnid > algae. This is probably because of the greater expense in running studies with fish, so there is greater value in avoiding these through use of a computational approach. They also identified the usefulness of compilations of QSAR estimates (e.g., the Danish QSAR database accessed via the ECB website[4], especially in the light of increasingly complex QSAR modelling approaches.

## 2.2 Methods used in this report

The utility of QSARs for the derivation of WFD EQS was examined by using currently prioritised and proposed substances as a case study. Metals and metalloids were excluded from analysis in this report because aquatic toxicity QSARs are unavailable for these substances in ECOSAR, and the utility of read-across for different salts of the same metal has been demonstrated in previous work (Worth and Patlewicz 2007).

Toxtree v1.02 (Ideaconsult, http://ecb.jrc.it/qsar/qsar-tools/index.php?c=TOXTREE) was used to assign a mode of action (MoA) class to each substance according to the Verhaar rules (Verhaar et al. 1992). Estimates of persistence, bioaccumulation and aquatic ecotoxicity were made with the EPI Suite v3.2 collection of QSARs (http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm). Specifically, BIOWIN (Boethling et al. 1994) was used to estimate persistence with the DT50 in days calculated according to criteria in the USEPA's PBT profiler[5], BCFWIN (Meylan et al. 1999) was used to estimate bioaccumulation, and ECOSAR (Meylan and Howard 1998) was used to estimate aquatic ecotoxicity. ECOSAR estimates were rejected if substance properties or aquatic toxicity predictions fell outside QSAR domains or above estimated solubility limits. Predicted log Kow results were used initially in this study although experimental log Kow

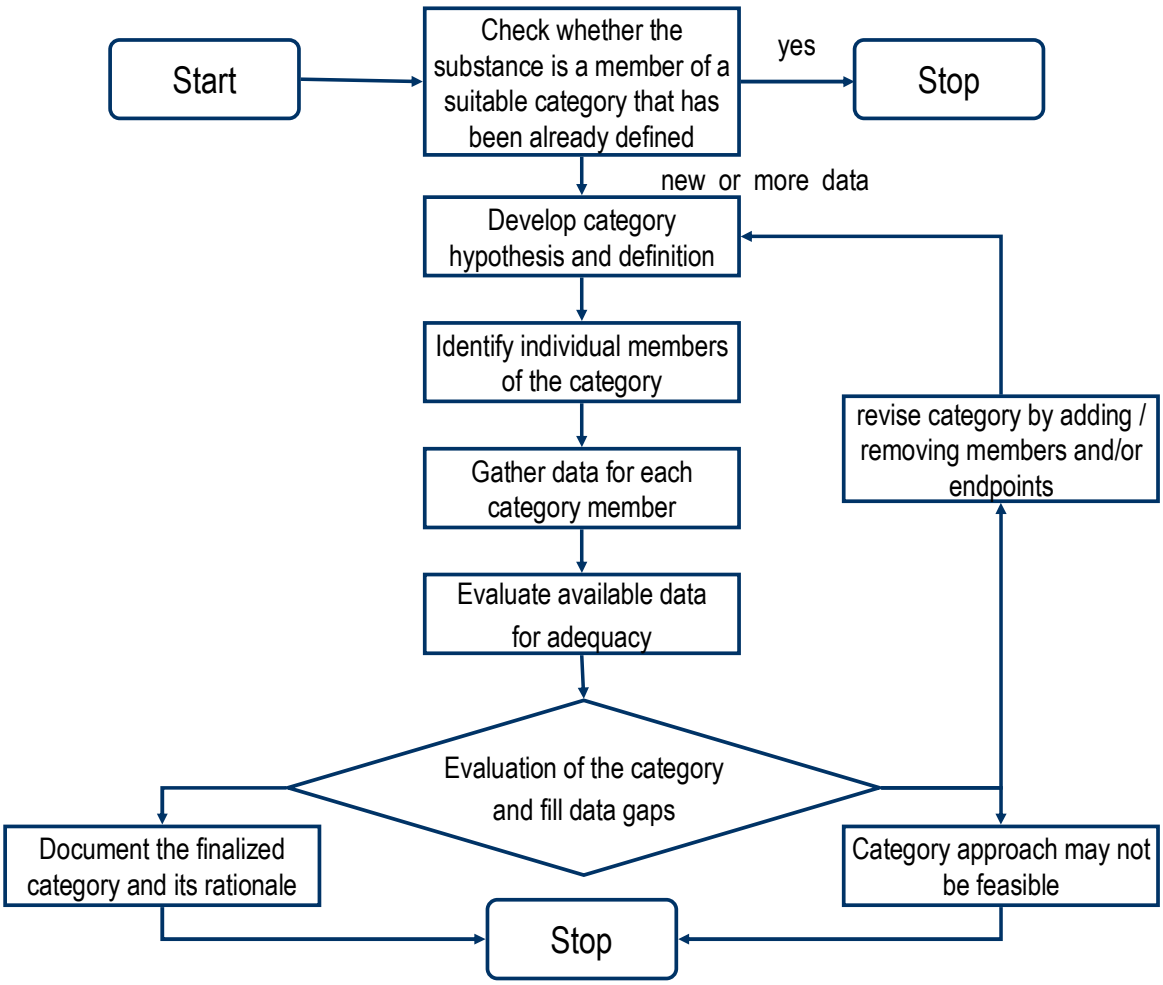
[4] http://ecbqsar.jrc.it/
[5] www.pbtprofiler.net

measurements would also normally be used if available. We chose to use predicted rather than experimental values in order to simulate the likely implications of QSAR use for EQS derivation if substances are proposed for EQS for which there are no experimental log Kow data.

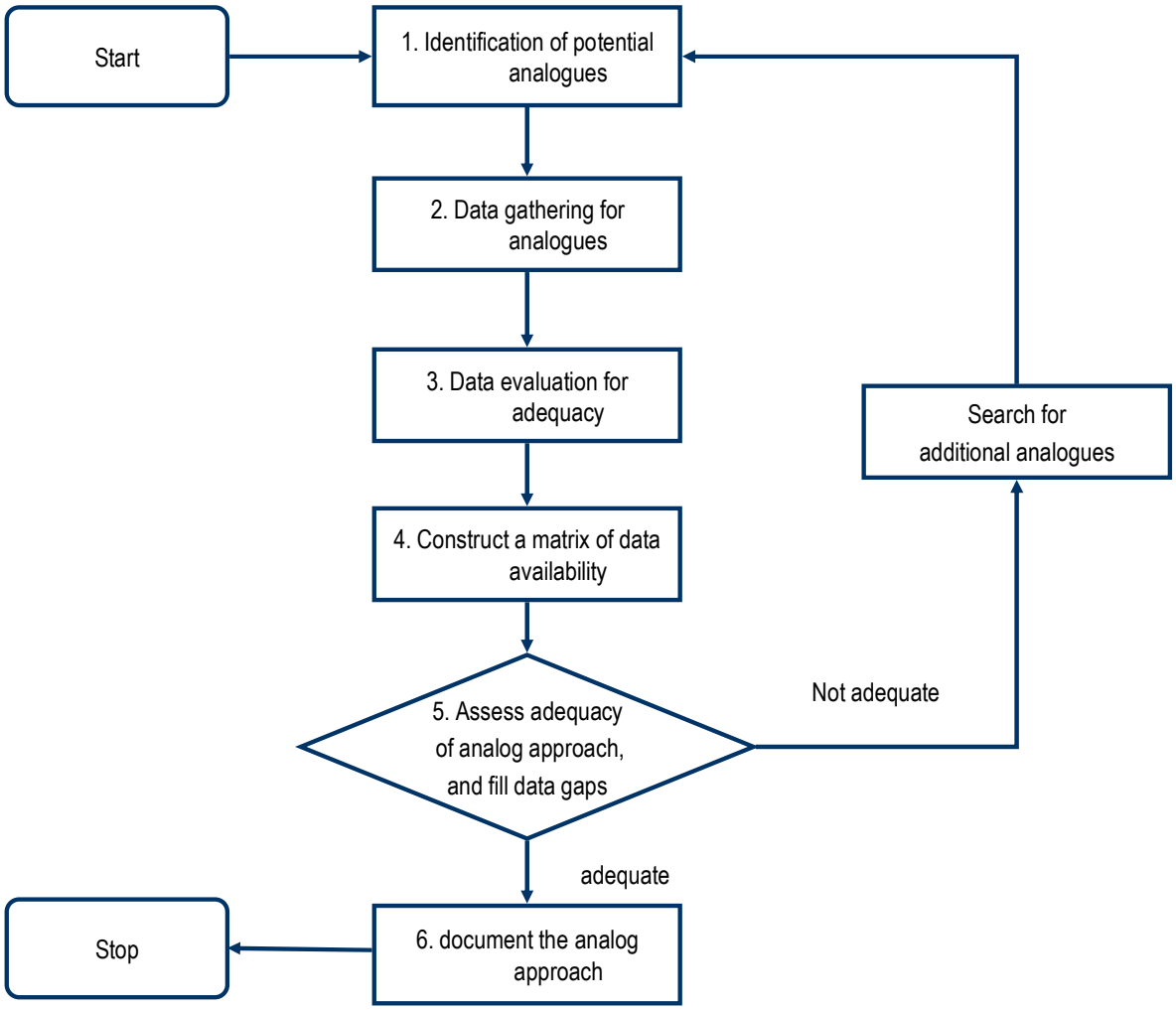
EQS for each substance based on measured ecotoxicity values were taken, where available, from reports to the European Commission by either the Fraunhofer Institute or INERIS[6]. These are subsequently referred to in this report as WFD data sheets. EQS based on QSAR estimates alone were calculated using the assessment factors in Lepper (2005) as if the QSAR estimates were themselves measured data.

Guidance on grouping of substances for read-across (OECD 2007b) was used to identify substances as potentially similar. Bassan and Worth (2008) recommend the use of a structured workflow when using non-testing approaches for hazard or risk assessment. OECD (2007b) also recommends using a structured process when deriving EQS based on QSARs or read-across to determine whether substances can be grouped into a category. The first step is to determine whether the individual substance or group of substances is already a named member of a category that has previously been evaluated. The category definition should then list all of the substances and endpoints covered. Although chemical structure is usually the starting point, a category definition could also refer to a group of chemicals related by a mode of action (e.g. non-polar narcotics) or a particular property. For each member of the category, relevant data should be gathered and evaluated for adequacy. A matrix of data availability (category endpoints vs. members) should be constructed with the category members arranged in a suitable order (e.g., according to log Kow). The ordering of the members should reflect any trends or progression seen within the category, and the cells of the matrix should indicate whether data are available or unavailable. This process is illustrated in the following chart from OECD (2007b):

[6] http://ec.europa.eu/environment/water/water-dangersub/pri_substances.htm




When there is an insufficient number of suitable analogues to develop a category, the analogue approach can be used, as shown in the following flow chart:




Seven PAHs selected as priority substances were used to illustrate the use of the category approach because of the availability of data for these substances, their identification as priority substances, and previous work on grouping of PAHs into categories in EC WFD substance datasheets. The seven substances were anthracene, fluoranthene, benzo-a-pyrene, benzo-b-fluoranthene, benzo-g,h,i-perylene, benzo-k-fluoranthene, and indeno(1,2,3-cd)pyrene. KOWWIN (Meylan and Howard 1995) was used to estimate log Kow for each of these substances.

The OECD QSAR Application Toolbox v1 was also used in this approach to identify suitable analogues from which to read across data to indeno(1,2,3-cd)pyrene. The target substance (indeno(1,2,3-cd)pyrene) was profiled mechanistically by superfragment, EcoSAR classification,

OASIS acute toxicity MOA, DNA binding, protein binding, organic functional groups, Cramer classification, and Verhaar classification.

Data from ecotoxicity tests were then gathered from the ECETOC, ECOTOX and OASIS aquatic databases. As expected, no data were available for indeno(1,2,3-cd)pyrene, so a category was defined on the basis of the EcoSAR classification (neutral organics), containing 2639 substances in the OECD toolbox database. Ecotoxicological data were gathered for these substances from the ECETOC, ECOTOX and OASIS aquatic databases and then sorted so that only the following data were used for gap filling: fish (any species) NOEC data for studies of >30d duration, 21-d *Daphnia magna* NOECs; and 72-h NOECs for any algal species. This produced 36 analogues for read-across to a chronic fish NOEC, 38 analogues for read-across to a *D. magna* chronic NOEC, and 11 analogues for read-across to a 72-h algal NOEC. Both the read-across (average value of five nearest neighbours) and trend analysis functions (using logKow as the predictor) in the OECD toolbox were used to fill ecotoxicity data gaps for indeno(1,2,3-cd)pyrene.

# 3. Results and discussion

## 3.1 Mode of action

A total of 61 organic substances remained after metals and metalloids were removed from the list (Table 3). Toxtree identified 13 as Class 1 MoA (narcosis/baseline toxicity), 3 as Class 2 MoA (less inert compounds), 14 as Class 3 MoA (unspecific reactivity), none as Class 4 MoA (compounds acting by a specific mechanism), and 31 as Class 5 MoA (not possible to classify). Hence the MoA of just over half of the substances prioritised by either the EC or the European Parliament could not be classified by the Verhaar rules, and less than a quarter of them were classified as operating via narcosis, which is the MoA for which most QSARs are currently available.

## 3.2 PBT/vPvB

Comparison was made against USEPA PBT criteria as shown below:

**Persistence**

| | Considered Persistent | Considered Very Persistent |
|---|---|---|
| Half-life in water, soil, and sediment | Half-life >= 2 months (>= 60 days) | Half-life > 6 months (> 180 days) |
| Half-life in Air | Half-life > 2 days | |

**Bioaccumulation**

| | Considered Bioaccumulative | Considered Very Bioaccumulative |
|---|---|---|
| Bioconcentration factor (BCF) | > = 1,000 | > 5,000 |

**Toxicity**

| | Low Concern | Moderate Concern | High Concern |
|---|---|---|---|
| Fish ChV (mg/l) | > 10 mg/l | 0.1 - 10 mg/l | < 0.1 mg/l |

These values were selected in preference to the REACH criteria below:

| **Criterion** | **PBT criteria** | **vPvB criteria** |
|---|---|---|
| P | Half-life > 60 d in marine water or > 40 d in fresh or estuarine water or half-life > 180 d in marine sediment or > 120 d in fresh or | Half-life > 60 d in marine fresh or estuarine water or > 180 d in marine, fresh or estuarine water; sediment or |

| Criterion | PBT criteria | vPvB criteria |
|---|---|---|
| | estuarine water; sediment or half-life in soil > 120 d | half-life in soil > 180 d |
| B | BCF > 2000 in fresh or marine aquatic species | BCF > 5000 |
| T | Chronic NOEC < 0.01 mg/l for fresh or marine water organisms, Category 1 or 2 carcinogen or mutagen or Category 1, 2 or 3 toxic for reproduction or chronically toxic (*i.e.* classified as T or Xn with R48) | Not applicable |

Note: (a) BCF is bioconcentration factor, NOEC is no-observed effect concentration and CMR is a substance classified as carcinogenic, mutagenic or toxic for reproduction
(b) For marine environmental risk assessment, half-life data in freshwater sediment can be overruled by data obtained under marine conditions
(c) Substances are classified when they fulfil the criteria for all three inherent properties for P, B and T. However, there is certain flexibility, for instance in cases where one criterion is marginally not fulfilled but the others are exceeded considerably.

The USEPA criteria were selected because thresholds for bioaccumulation and toxicity are less stringent than those under REACH, which seems appropriate for a QSAR-based screening approach. Also, the WFD does not currently identify PBT thresholds (Pavan and Worth 2006).

Predictions that a substance was either PBT or vPvB agreed with conclusions from measured data in 35 of 41 cases (85.4%) where measured data were available for comparison (Table 3). Of these, 29.4% (10) were judged to be PBT or vPvB, and 71.4% (25) were judged to be not PBT or vPvB. There were 20 cases where there were insufficient measured data for a comparison. There was disagreement between estimated and measured PBT properties in six of 42 cases (14.3%). In five cases QSAR estimates identified a substance as not PBT when measured data suggest that it is PBT (hexachlorobutadiene, hexachlorocyclohexane, trichlorobenzenes, musk ketone and musk xylene), and in one case a substance was identified as PBT through QSAR estimates when measured data suggest that it may not be PBT (trifluralin). Each of these substances is discussed below:

- Hexachlorobutadiene (MoA Class 5): The European Chemical Substances Information System (ESIS[7]) defines hexachlorobutadiene as PBT, vPvB and POP. BIOWIN identifies hexachlorobutadiene as persistent and ECOSAR identifies acute toxicity (chronic estimates are unavailable from ECOSAR, but an assessment factor of 10 on the acute estimate would produce a value that fulfils the T criterion in PBT). However BCFWIN underestimates hexachlorobutadiene's bioaccumulation potential, with an estimated BCF of 960 (based on a KOWWIN log Kow estimate of 4.72), when a measured fish BCF of 17000 is reported in a risk assessment by Euro Chlor (2002). A BCF of 960 just falls short of the USEPA threshold of 1000 for identifying a substances as potentially bioaccumulative. Experimental Kow values of 4.78-4.9 are reported for hexachlorobutadiene in its WFD substance datasheet. Dearden and Worth (2007) recommend that two or more software programs are used to estimate log Kow, so when estimates of BCF did not exceed the B criterion we used VCCLAB[8] to provide estimates of log Kow from several available models. Estimated log Kow values across these software programs reported by VCCLAB ranged from 3.87 to 6.17 (average = 4.78). If a value of 4.9 had been used in BCFWIN the resulting BCF would be 1183, which exceeds the USEPA threshold and would have identified hexachlorobutadiene as

[7] http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=pbt
[8] www.vcclab.org

potentially PBT. Use of the average log Kow value calculated by VCCLAB across several software programs would still have produced a BCF that fell just short of this threshold.

- Hexachlorocyclohexanes (MoA Class 1): ESIS identifies lindane (a hexachlorocyclohexane) as a POP, but not as PBT or vPvB. BIOWIN identifies lindane as persistent, but BCFWIN estimates a BCF of only 310 (based on a KOWWIN log Kow estimate of 4.26), and ECOSAR estimates a fish chronic value of 0.3 mg $l^{-1}$ (lower than estimates for invertebrate or algal toxicity), neither of which exceed B or T thresholds. Measured fish BCFs for hexacyclohexanes and lindane reported in the WFD substance datasheet range from 210 to 2400, and data for chronic toxicity to fish suggest a value nearer to 0.003 mg $l^{-1}$, with even lower values for insects (0.0002 mg $l^{-1}$ for mayflies). Experimentally derived log Kow values range from 3.5 to 3.85, and estimated log Kow values across several software programs reported by VCCLAB range from 3.53 to 4.26 (average = 3.84), which are not higher than the KOWWIN estimate of 4.26, so their use does not increase estimates of BCF or toxicity.

- Trichlorobenzenes (MoA Class 1): ESIS does not include an entry for trichlorobenzenes. BIOWIN identifies trichlorobenzenes as persistent and ECOSAR estimates a chronic fish toxicity of 0.036 mg $l^{-1}$, which exceeds the USEPA T criterion. However, BCFWIN estimates a BCF of 340 which does not exceed the B criterion; fish BCF data in the WFD datasheet range from 120-8400 over all isomers, with a considerable range within each isomer. KOWWIN estimates a log Kow of 3.93 for trichlorobenzenes, but measured values appear to be higher than this, ranging up to 4.49 for 1,3,5-trichlorobenzene. If this experimental value is used in BCFWIN it results in a BCF of 571.9, which still does not exceed the B criterion. Estimated log Kow values across several software programs reported by VCCLAB for 1,3,5-trichlorobenzene range from 3.67 to 4.08 (average = 3.88).

- Musk ketone (MoA Class 3): ESIS does not include an entry for musk ketone. BIOWIN and ECOSAR identify musk ketone as P (DT50=60 d) and T (fish chronic toxicity = 0.006 mg $l^{-1}$), but BCFWIN estimates a BCF of 60, when EC (2005a) reports a measured BCF of 1380. The log Kow estimated by KOWWIN and the maximum measured log Kow are both 4.3 (EC 2005a), so use of an experimental log Kow in BCFWIN does not increase the BCF estimate. VCCLAB does not have an entry for musk ketone, but a log Kow of 4.71 is estimated by SPARC[9], which if used in BCFWIN would produce an estimated BCF of 122, which still does not exceed the B criterion.

- Musk xylene (MoA Class 3): ESIS states that musk xylene is currently under evaluation, and INERIS report that P, B and T criteria appear to be fulfilled and that an EC decision on this is imminent. BIOWIN and ECOSAR identify musk xylene as P (DT50=180 d) and T (fish chronic toxicity = 0.005 mg $l^{-1}$), but BCFWIN estimates a BCF of 530, when EC (2005b) reports a measured BCF of 4400. However, if the measured log Kow of 4.9 reported by EC (2005b) is used in BCFWIN instead of the KOWWIN estimated value 0f 4.45 it results in an estimated BCF of 1183, which exceeds the B criterion. VCCLAB does not have an entry for musk xylene, but a log Kow of 5.43 is estimated by SPARC, which if used in BCFWIN would produce an estimated BCF of 3028, which also exceeds the B criterion.

- Trifluralin (MoA Class 3): ESIS does not include an entry for trifluralin, but this substance is identified as PBT by the USEPA final rule and is also a UNEP POP (because of Long Range

[9] http://ibmlc2.chem.uga.edu/sparc

Transport). However, despite this and although BIOWIN, BCFWIN and ECOSAR identify trifluralin as PBT, measured data on persistence reported in the WFD substance datasheet suggest a worst case DT50 in water of 13 days, which does not meet the minimum P criterion.

In summary, for this set of substances BIOWIN, BCFWIN and ECOSAR were mostly successful in accurately identifying them as PBT or vPvB when measured data were available for comparison. On the six occasions when they were unsuccessful, there were five false negatives and one possible false positive. Two of these substances are either currently classified as PBT ((hexachlorobutadiene) or likely to become so in the near future (musk xylene) and would have been correctly identified as PBT if experimental log Kow values had been used in place of KOWWIN estimates (or if the SPARC estimate had been used for musk xylene). Three of the substances (hexachlorocyclohexanes, trichlorobenzenes and musk ketone) were not identified as PBT by QSAR estimates when measured data suggest that they potentially fulfil PBT criteria. However, these substances are not currently classified as PBT in either the USA or the European Union so their status is uncertain. The final substance (trifluralin) was identified as PBT by QSAR estimates and is regarded as such by USEPA, although this is not the case in the European Union. It would therefore appear that this apparent false positive result is a borderline case which clearly exceeds B and T criteria, but may not be sufficiently persistent to exceed the P criterion.

The previously known tendency for BCFWIN to underestimate bioaccumulation (Pavan et al. 2006) was apparent in this assessment.

### 3.3 Relationships between ECOSAR acute and chronic estimates and measured data for fish, invertebrates and algae

Figures 1-6 show plots of available ECOSAR estimates and measured values for fish, daphnids and algae for both acute and chronic exposures. Plots are shown for all matched estimated and measured values, plus values for MoA Classes 1, 3, and 5 (there were insufficient values for Class 2 and no values for Class 4).The diagonal line in each plot shows the one to one relationship, so all values falling below this line are for substances where the ECOSAR estimate of toxicity was lower than the measured value. If an EQS were to be derived from such an ECOSAR estimate it would be less stringent than one based on the equivalent measured value and therefore potentially less protective.

The plots show that for these prioritised and proposed substances, ECOSAR generally underpredicted toxicity, with the exception of daphnid and algal chronic results for MoA class 1 (narcosis) and daphnid chronic results for class 3 (unspecific reactivity). This means that in most cases an EQS for one of these substances based on ECOSAR-estimated toxicity for any of the three trophic levels will most likely be less stringent than one based on measured data, as we show in the next section.

### 3.4 Environmental Quality Standards

Figure 7 shows the ratios between EQSs based on ECOSAR estimates and measured data for those substances (n=44) where it was possible to calculate EQSs according to the procedure followed in the EU (Lepper 2005, EC 2003).

Use of ECOSAR estimates alone when deriving PNECs led to more stringent (i.e. lower) EQS values for 13 substances (29%), and to less stringent (i.e. higher) EQS values for 31 substances (71%). Approximately half (48%) of the EQS based on QSAR estimates were within a factor of 10 of the EQS based on measured data. However, for several substances, including some from each of MoA classes 1, 3 and 5, EQS based on ECOSAR estimates alone differed from those based on measured values by more than a hundred-fold. Such estimates would therefore be an unreliable sole basis for setting EQS.

**Table 3** **Mode of action (MOA) class, SMILES notation and Log Kow (KOWWIN estimates) for proposed EQS analysed in this report. MoA: 1 = narcosis/baseline toxicity, 2 = less inert compounds, 3 = unspecific reactivity, 4 = compounds/groups acting by a specific mechanism, 5 = not possible to classify. N/D = no data. Greyed entries indicate a mismatch between estimated and measured PBT.**

| Substances | MoA Class | SMILES Notation | Log KoW (KOWWIN estimates) | Estimated Persistence (Surface water DT50 days) | Estimated BCF | Toxicity (fish ChV mg $l^{-1}$ unless otherwise stated) | Estimates indicate PBT/ vPvB? | Measured values indicate PBT/ vPvB? |
|---|---|---|---|---|---|---|---|---|
| Alachlor | 3 | CCc1cccc(CC)c1N(COC)C(=O)CCl | 3.37 | 60 | 100 | 0.031 | No | No |
| Anthracene | 1 | c(c(ccc1)cc(c2ccc3)c3)(c1)c2 | 4.35 | 60 | 530 | 0.16 | No | No |
| Atrazine | 5 | n(c(nc(n1)NC(C)C)NCC)c1Cl | 2.82 | 60 | 9.8 | 4 | No | No |
| Benzene | 1 | c(cccc1)c1 | 1.99 | 38 | 8.7 | 7.6 | No | No |
| Pentabromo-diphenylether | 5 | Brc1cc(c(cc1Oc2c(cc(cc2)Br)Br)Br)Br | 7.66 | 180 | 8100 | 0.00064 | Yes | Yes |
| $C_{10-13}$ Chloroalkanes (1-chloro-decane, CAS 822-13-9, selected as example for PBT analysis) | 1 | CLCCCCCCCCCCCCC | 6.98 | 15 (140 in sediment) | 1500 | 0.00097 | Yes | Yes |
| Chlorfenvinphos | 5 | CCOP(=O)(OCC)OC(=CCl)c1ccc(Cl)cc1Cl | 4.15 | 60 | 28 | Not estimated (but no ECOSAR estimates <0.1) | No | No |
| Chlorpyrifos | 5 | CCOP(=S)(OCC)Oc1nc(Cl)c(Cl)cc1Cl | 4.66 | 180 | 1300 | Not estimated (but no ECOSAR estimates <0.1) | No | No |
| 1,2-Dichloroethane | 1 | ClCCCl | 1.83 | 38 | 2.8 | 13 | No | No |
| Dichloromethane | 1 | ClCCl | 1.34 | 38 | 1.8 | 30 | No | No |
| DEHP | 5 | O=C(OCC(CCCC)CC)c(c(ccc1)C(=O)OCC(CCCC)CC)c1 | 8.39 | 15 | 310 | Not estimated (but ECOSAR estimates <0.01) | No | No |
| Diuron | 5 | O=C(N(C)C)Nc(ccc(c1Cl)Cl)c1 | 2.67 | 38 | 23 | 5.8 | No | No |
| Endosulfan | 3 | ClC2=C(Cl)C3(Cl)C1COS(=O)OCC1C2(Cl)C3(Cl)Cl | 3.5 | 180 | 180 | Not estimated (but acute fish = 0.87) | No | No |
| Fluoranthene | 1 | c(c(ccc1)ccc2)(c1c(c3ccc4)c4)c23 | 4.93 | 60 | 1900 | 0.055 | Yes | Yes |
| Hexachlorobenzene | 1 | c(c(c(c(c1Cl)Cl)Cl)Cl)(c1Cl)Cl | 5.86 | 180 | 5200 | 0.012 | Yes | Yes |
| Hexachlorobutadiene | 5 | C(=C(C(=C(Cl)Cl)Cl)Cl)(Cl)Cl | 4.72 | 180 | 960 | 0.089 (acute) | No | Yes |
| Hexachlorocyclohexane | 1 | ClC1C(Cl)C(Cl)C(Cl)C(Cl)C1Cl | 4.26 | 180 | 310 | 0.3 | No | Yes |
| Isoproturon | 5 | CC(C)c1ccc(NC(=O)N(C)C)cc1 | 2.84 | 38 | 32 | 3.7 | No | No |
| Napthalene | 1 | c(c(ccc1)ccc2)(c1)c2 | 3.17 | 38 | 69 | 1.2 | No | No |

| Substances | MoA Class | SMILES Notation | Log KoW (KOWWIN estimates) | Estimated Persistence (Surface water DT50 days) | Estimated BCF | Toxicity (fish ChV mg $l^{-1}$ unless otherwise stated) | Estimates indicate PBT/ vPvB? | Measured values indicate PBT/ vPvB? |
|---|---|---|---|---|---|---|---|---|
| Nonylphenol | 5 | Oc1ccc(cc1)CCCCCCCCC | 5.99 | 15 | 540 | 0.004 | No | No |
| Octylphenol | 2 | Oc(ccc(c1)CCCCCCCC)c1 | 5.5 | 15 | 340 | 0.007 | No | No |
| Pentachlorobenzene | 1 | c(c(c(c(c1Cl)Cl)Cl)Cl)(c1)Cl | 5.22 | 180 | 1900 | 0.038 | Yes | Yes |
| Pentachlorophenol | 5 | Oc(c(c(c(c1Cl)Cl)Cl)Cl)c1Cl | 4.74 | 180 | 700 | 0.019 | No | No |
| Benzo(a)pyrene | 1 | c(c(c(cc1)ccc2)c2cc3)(c3cc(c4ccc5)c5)c14 | 6.11 | 60 | 10000 | 0.006 | Yes | N/D |
| Benzo(b)fluoranthene | 5 | c12ccccc1cc3c4ccccc4c5c3c2ccc5 | 6.11 | 60 | 5600 | 0.006 | Yes | N/D |
| Benzo(k)fluoranthene | 5 | c2ccc1cc3c(cc1c2)c4cccc5cccc3c45 | 6.11 | 60 | 10000 | 0.006 | Yes | N/D |
| Benzo(g,h,i)perylene | 5 | c16cccc2ccc3ccc4ccc5cccc6c5c4c3c12 | 6.7 | 60 | 25000 | 0.002 | Yes | N/D |
| Indeno(1,2,3-cd)pyrene | 5 | c(c(c(c(ccc1)c2)c1cc3)c3cc4)(c2c(c5ccc6)c6)c45 | 6.7 | 60 | 29000 | 0.002 | Yes | N/D |
| Simazine | 5 | n(c(nc(n1)NCC)NCC)c1Cl | 2.4 | 60 | 4.6 | 8.6 | No | No |
| Trichlorobenzenes | 1 | Clc1cc(cc(c1)Cl)Cl | 3.93 | 60 | 340 | 0.36 | No | Yes |
| Trichloromethane | 1 | C(Cl)(Cl)Cl | 1.52 | 38 | 6.6 | 30 | No | No |
| Trifluralin | 3 | CCCN(CCC)c1c(cc(cc1N(=O)=O)C(F)(F)F)N(=O)=O | 5.31 | 180 | 2600 | 0.003 | Yes | No |
| Perfluorooctane sulphonic acid | 5 | O=S(=O)(O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F | 6.28 | 180 | 56 | 0.09 | No | N/D |
| Perfluorooctanoic acid (PFOA) | 3 | O=C(O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F | 6.3 | 180 | 56 | 0.072 | No | N/D |
| Ammonium perfluorooctanoate | 3 | OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F | 6.3 | | | | N/D | N/D |
| Amidotrizoate | 5 | CC(=O)Nc1c(I)c(C(=O)O)c(I)c(NC(=O)C)c1I | 1.37 | 180 | 3.2 | 2100 | No | N/D |
| AMPA (glyphosate metabolite) | 5 | O=P(CN)(O)O | -2.17 | 15 | 3.2 | Not estimated (but no ECOSAR estimates <0.1) | No | N/D |
| Bentazon | 3 | O=C(N(S(=O)(=O)Nc1cccc2)C(C)C)c12 | 1.67 | 38 | 13 | 44 | No | No |
| Bisphenol A | 2 | Oc(ccc(c1)C(c(ccc(O)c2)c2)(C)C)c1 | 3.64 | 38 | 72 | 0.05 | No | No |
| 4 4'-biphenol | 2 | Oc(ccc(c(ccc(O)c1)c1)c2)c2 | 2.8 | 15 | 28 | 0.098 | No | N/D |
| Carbamazepine | 5 | NC(=O)N2c1ccccc1C=Cc3ccccc23 | 2.25 | 38 | 15 | 14 | No | N/D |
| Clotrimazole | 5 | Clc1ccccc1C(c2ccccc2)(c3ccccc3)n4ccnc4 | 6.26 | 60 | 13000 | Not estimated (but Daphnia acute = 0.023) | Yes | N/D |
| Dibutylpthalate | 3 | O=C(OCCCC)c(c(ccc1)C(=O)OCCCC)c1 | 4.61 | 8.7 | 580 | 0.11 | No | No |
| Diclofenac | 5 | OC(=O)Cc1ccccc1Nc2c(Cl)cccc2Cl | 4.02 | 38 | 3.2 | 4.9 | No | N/D |
| Dicofol | 3 | C(c1ccc(Cl)cc1)(c2ccc(Cl)cc2)C(Cl)(Cl)Cl | 5.81 | 180 | 1500 | Not estimated (but | Yes | Yes |

| Substances | MoA Class | SMILES Notation | Log KoW (KOWWIN estimates) | Estimated Persistence (Surface water DT50 days) | Estimated BCF | Toxicity (fish ChV mg $l^{-1}$ unless otherwise stated) | Estimates indicate PBT/ vPvB? | Measured values indicate PBT/ vPvB? |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Daphnia acute = 0.00008) | | |
| DTPA | 5 | O=C(O)CN(CCN(CC(=O)O)CC(=O)O)CCN(CC(=O)O)CC(=O)O | -4.91 | 8.7 | 3.2 | Not estimated (but algal ChV = 6850) | No | N/D |
| EDTA | 5 | O=C(O)CN(CCN(CC(=O)O)CC(=O)O)CC(=O)O | -3.86 | 8.7 | 3.2 | Not estimated (but algal ChV = 2269) | No | No |
| ETBE | 5 | O(C(C)(C)C)CC | 1.92 | 15 | 6 | 11 | No | N/D |
| Glyphosate | 5 | OC(=O)CNCP(O)(O)=O | -4.47 | 15 | 3.2 | Not estimated (but algal ChV = 2099) | No | No |
| HHCB | 5 | O(CC(c(c1cc(c2C(C3C)(C)C)C3(C)C)c2)C)C1 | 6.26 | 60 | 13000 | 0.005 | Yes | Yes |
| Iopamidol | 5 | CC(O)C(=O)Nc1c(I)c(C(=O)NC(CO)CO)c(I)c(C(=O)NC(CO)CO)c1I | -1.38 | 60 | 3.2 | 65000 | No | N/D |
| Mecoprop | 5 | O=C(O)C(Oc(c(cc(c1)Cl)C)c1)C | 2.94 | 38 | 3.2 | 31 | No | No |
| 4-methylbenzylidene camphor | 3 | O=C(C(C(C1(C)C)CC2)=Cc(ccc(c3)C)c3)C12C | 5.92 | 60 | 7200 | 0.008 | Yes | N/D |
| Musk ketone | 3 | O=C(c(c(c(N(=O)=O)c(c1N(=O)=O)C(C)(C)C)C)c1C)C | 4.31 | 60 | 60 | 0.006 | No | Yes |
| Musk xylene | 3 | O=N(=O)c(c(c(N(=O)=O)c(c1N(=O)=O)C(C)(C)C)C)c1C | 4.45 | 180 | 530 | 0.005 | No | Yes |
| MTBE | 5 | O(C(C)(C)C)C | 1.43 | 15 | 3.2 | 26 | No | No |
| Napthalene-1,5-disulfonate | 3 | O=S(=O)(O)c(c(c(c(S(=O)(=O)O)cc1)cc2)c1)c2 | -0.94 | 15 | 3.2 | 99000 | No | N/D |
| Octylmethoxycinnamate | 3 | O=C(OCC(CCCC)CC)C=Cc(ccc(OC)c1)c1 | 5.8 | 15 | 5900 | 0.003 | Yes | N/D |
| Quinoxyfen (5,7-dichloro-4-(p-fluorophenoxy)quinoline | 5 | c1c(Cl)cc2nccc(Oc3ccc(F)cc3)c2c1Cl | 5.69 | 180 | 4800 | 0.018 | Yes | Yes |
| Tetrabromobisphenol A (TBBP-A) | 5 | Oc(c(cc(c1)C(c(cc(c(O)c2Br)Br)c2)(C)C)Br)c1Br | 7.2 | 180 | 14000 | 0.003 | Yes | Yes |
| Tonalid (AHTN) | 3 | O=C(c(c(cc(c1C(CC2C)(C)C)C2(C)C)C)c1)C | 6.35 | 60 | 2200 | 0.004 | Yes | Yes |

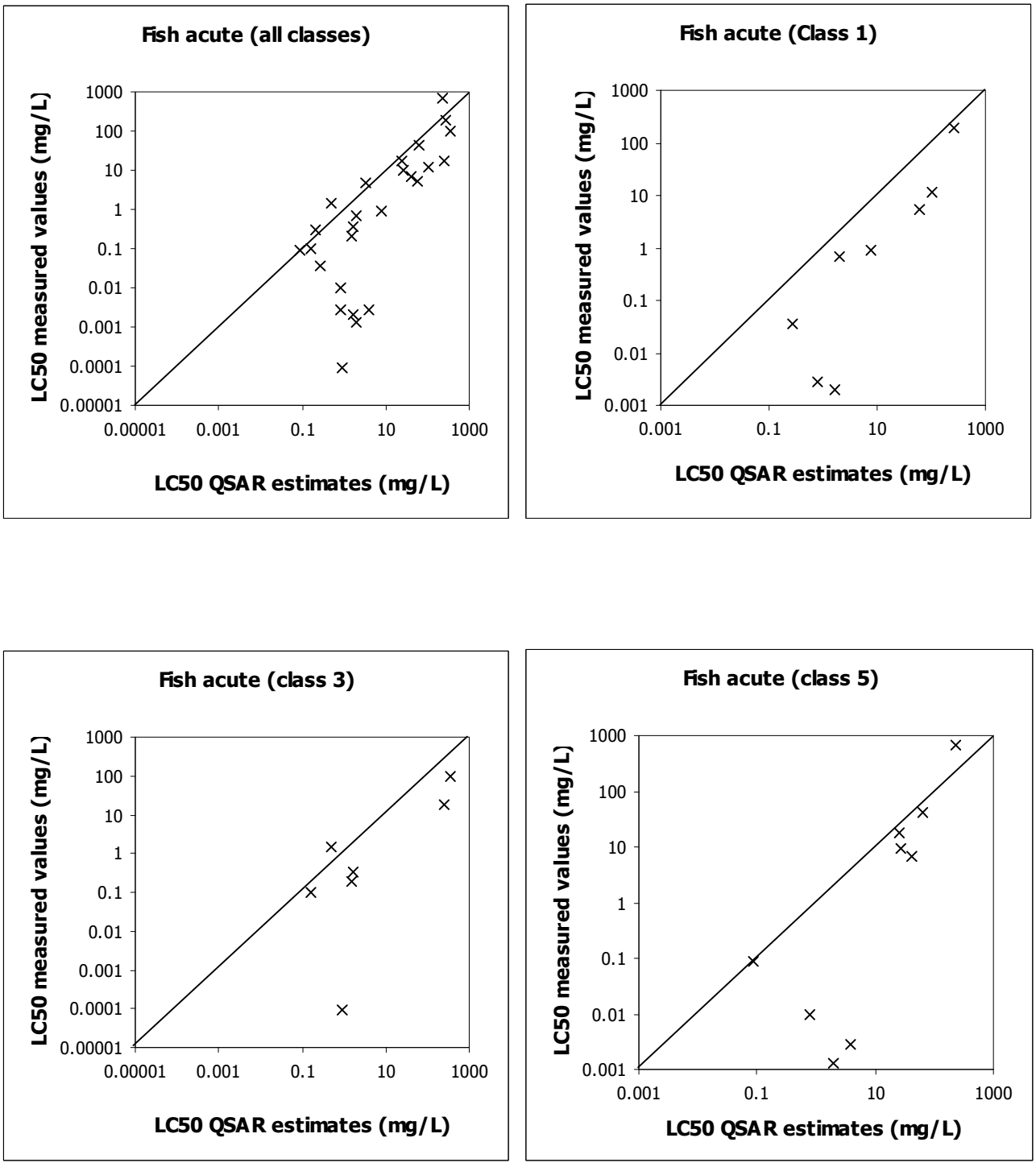



**Figure 1** **Relationship between ECOSAR estimates and measured values for fish acute toxicity.**

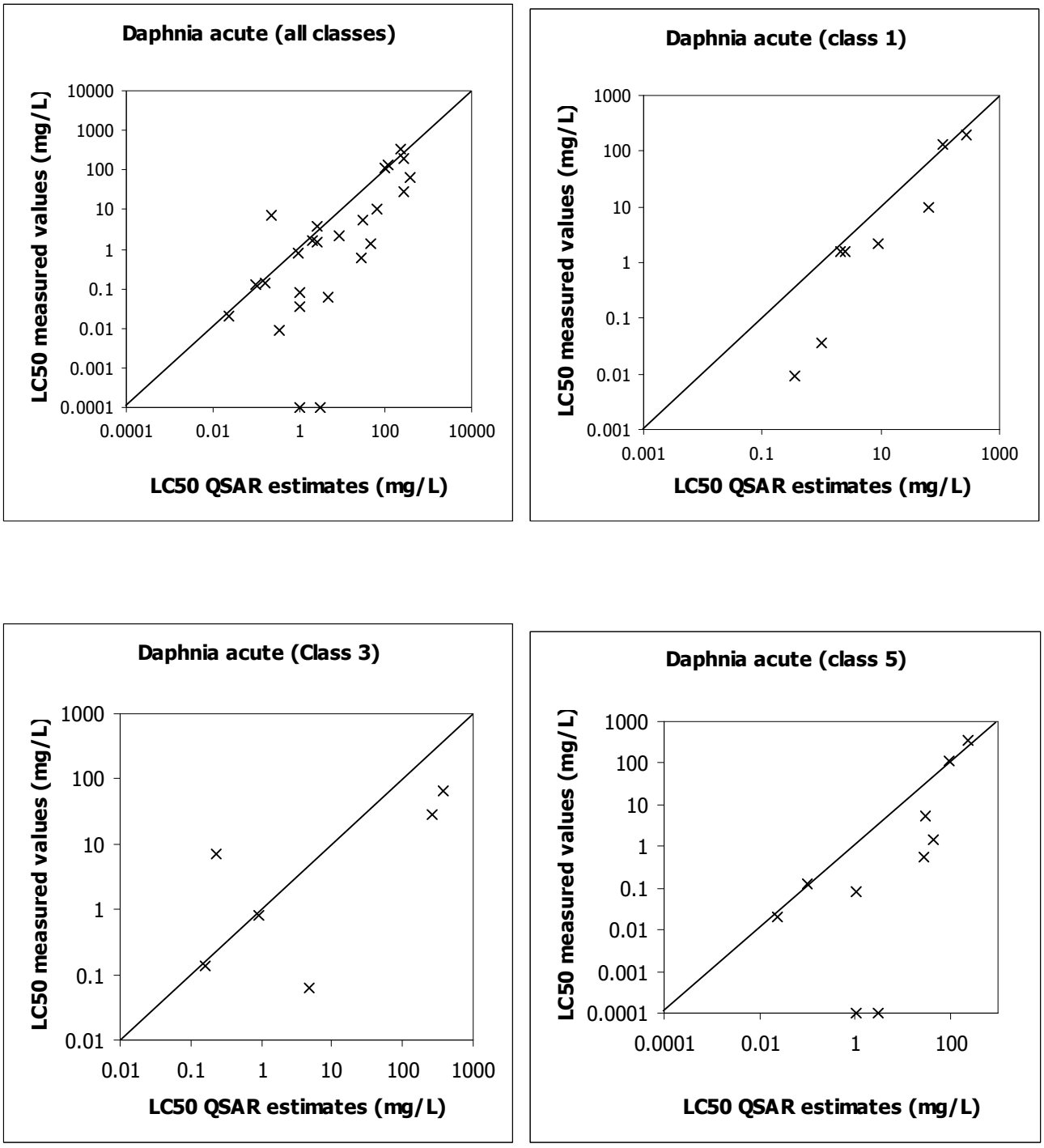



**Figure 2** **Relationship between ECOSAR estimates and measured values for daphnid acute toxicity.**

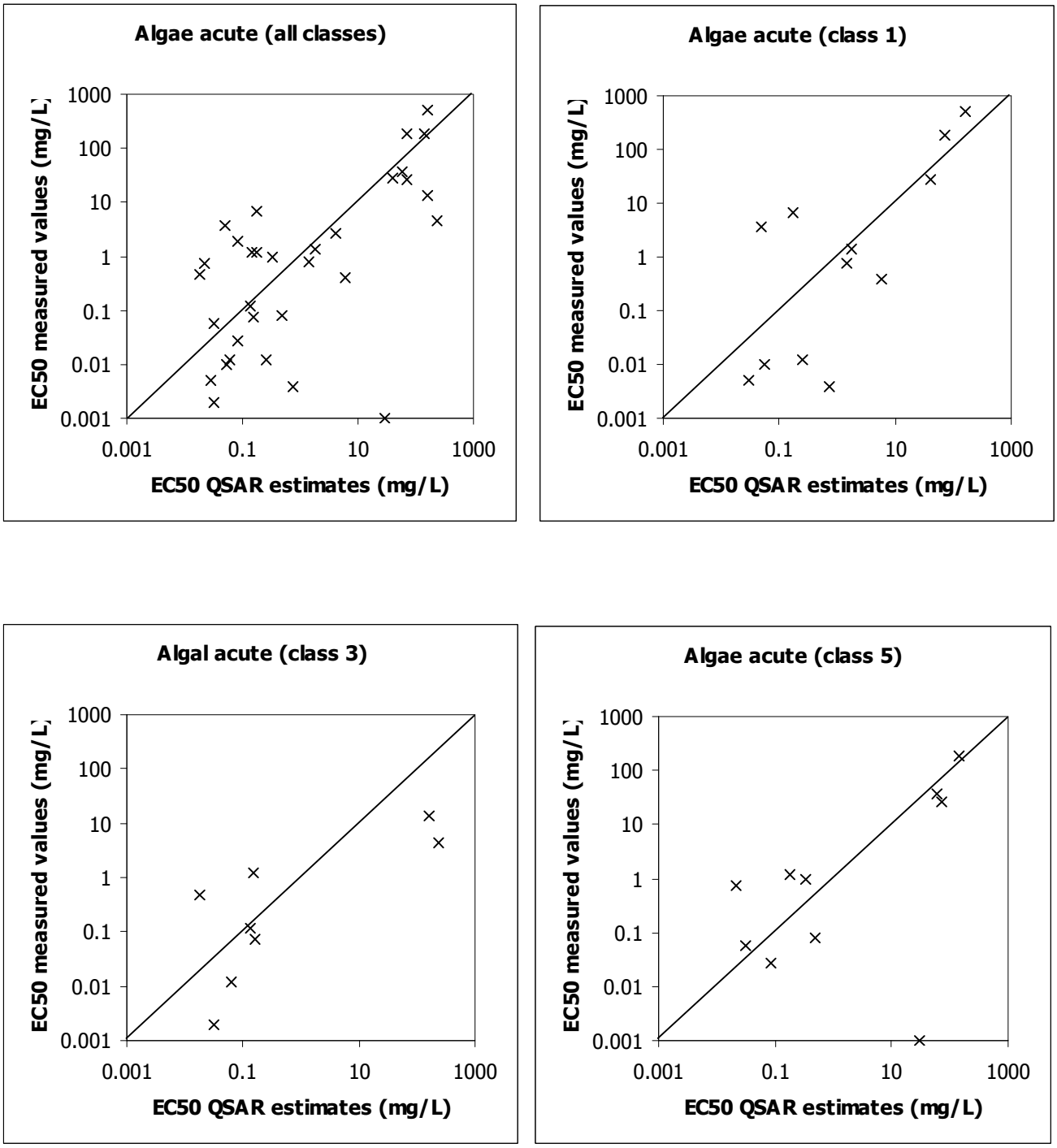
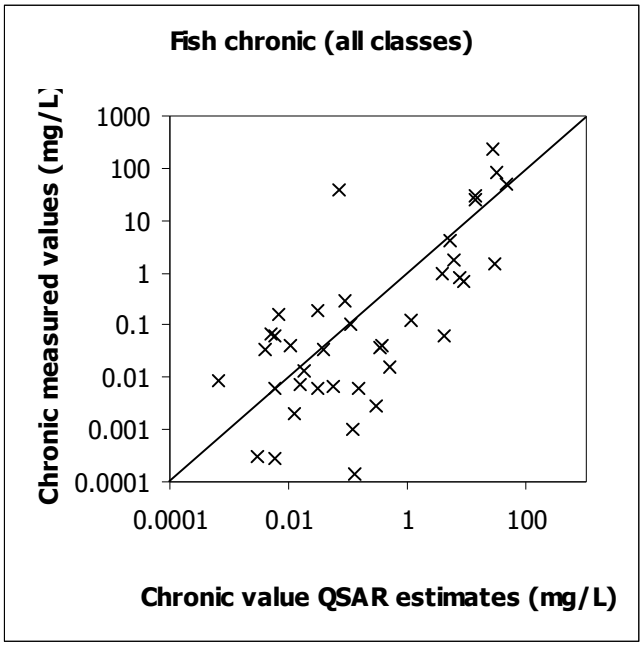
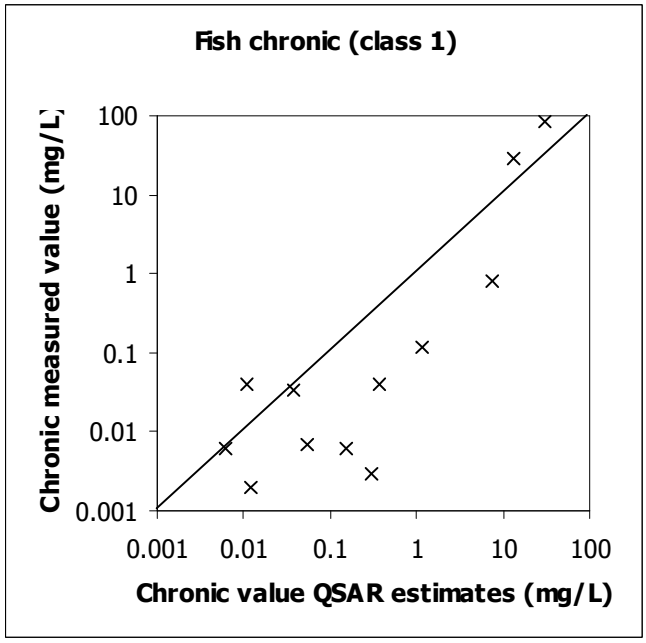
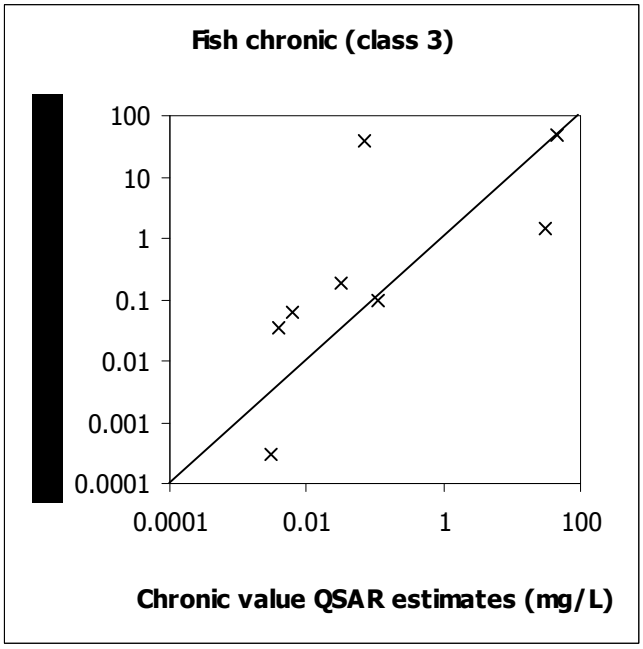



**Figure 3** **Relationship between ECOSAR estimates and measured values for algal acute toxicity.**



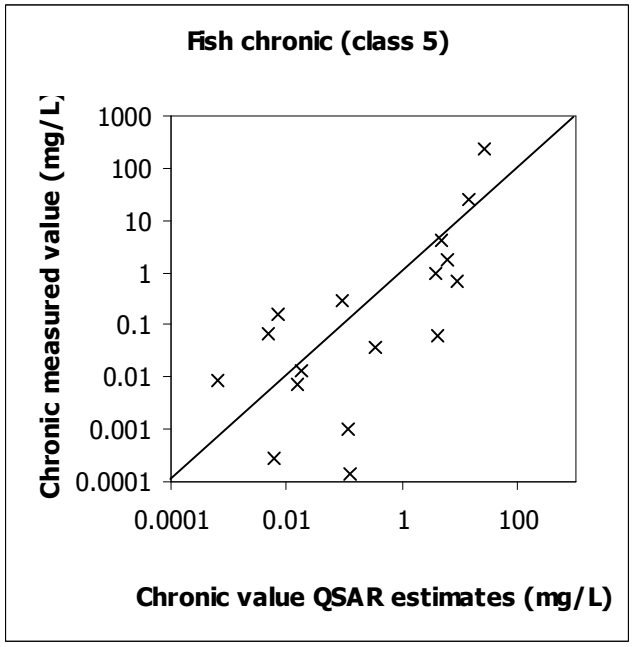




**Figure 4** **Relationship between ECOSAR estimates and measured values for fish chronic toxicity.**

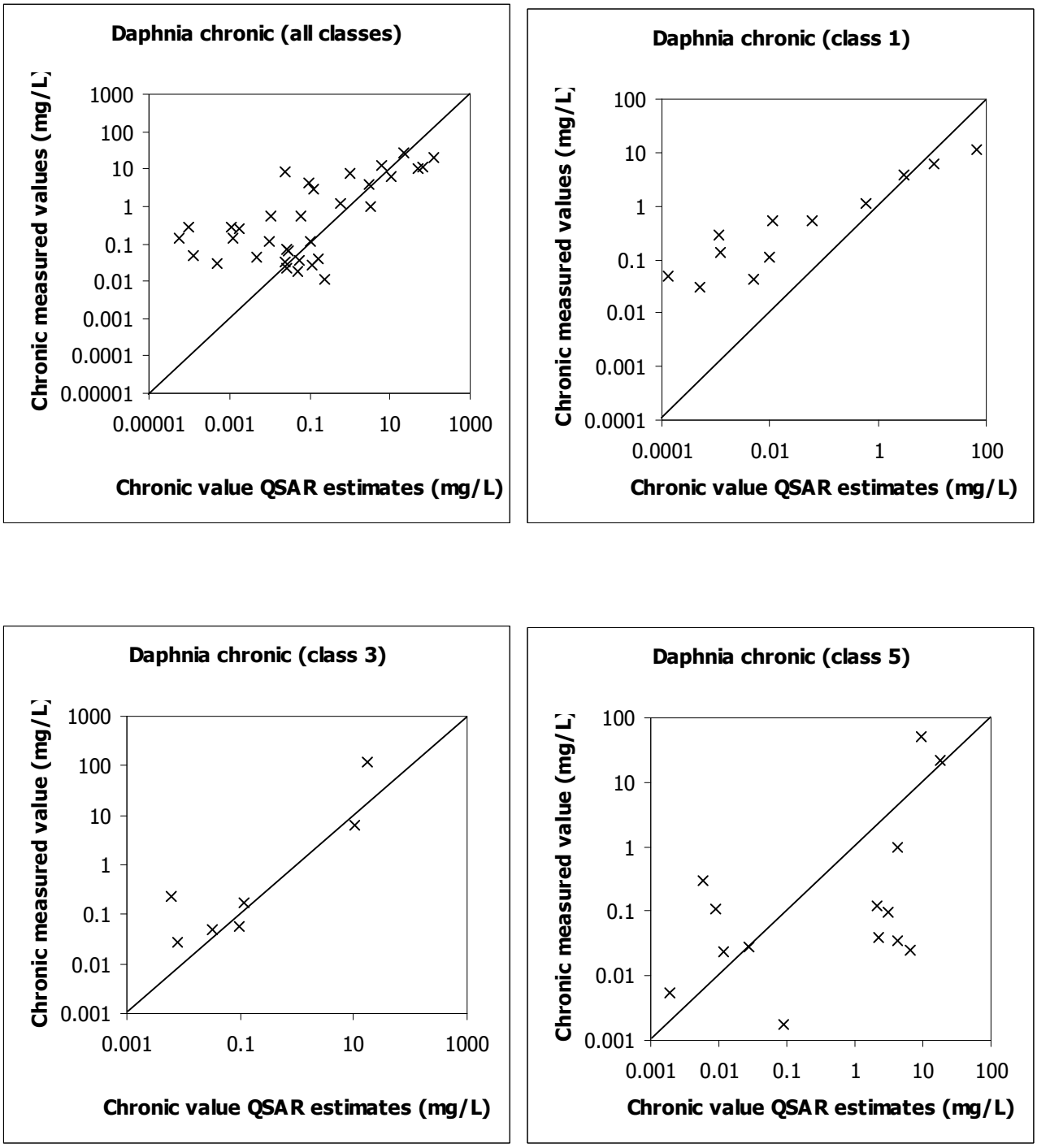



**Figure 5** **Relationship between ECOSAR estimates and measured values for daphnid chronic toxicity.**

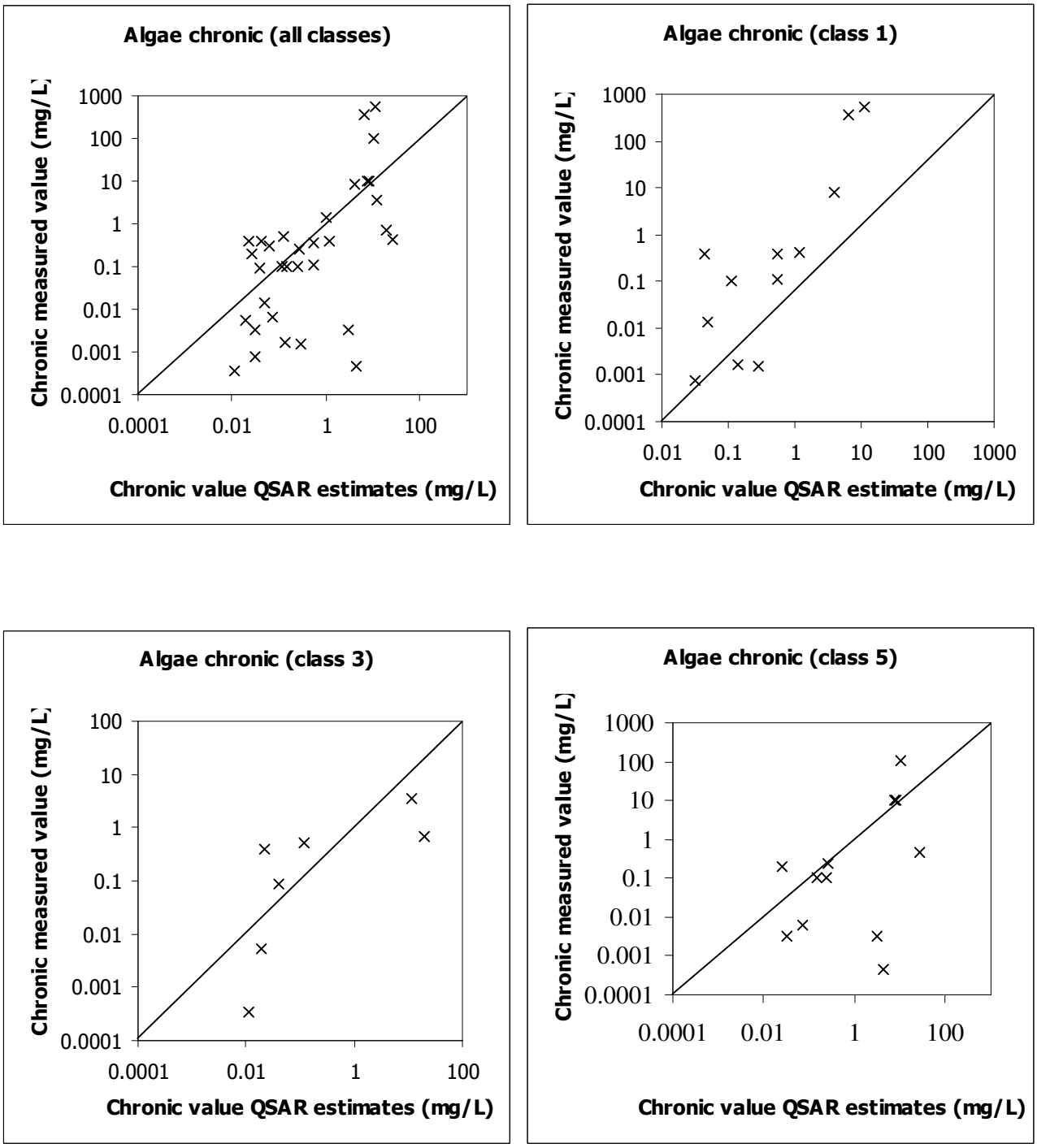



**Figure 6** **Relationship between ECOSAR estimates and measured values for algal chronic toxicity.**

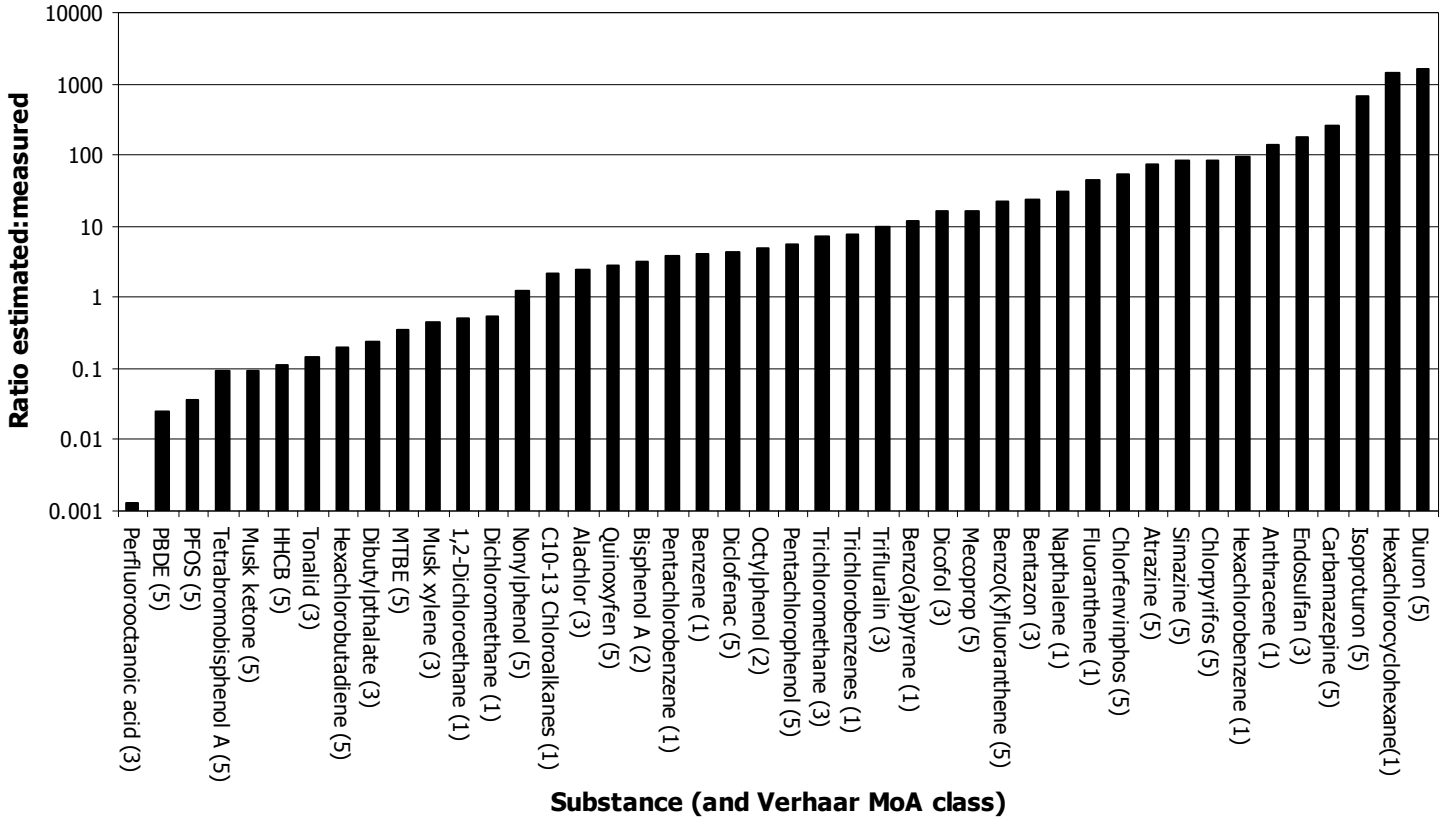



**Figure 7 Ratio of freshwater EQS based on QSAR estimates to EQS based on measured values.**

## 3.5 Most sensitive trophic level

The results above suggest that it would be unwise to rely solely on ECOSAR estimates when deriving an EQS. However, can these estimates be used to identify the most sensitive trophic group (fish, invertebrates or algae) so that data gaps can be filled efficiently by directing testing towards the most sensitive trophic group?

There were 18 substances for which complete base set ECOSAR estimates and measured data were available for acute toxicity to fish, daphnids and algae. ECOSAR predicted the most sensitive trophic group in seven of 18 cases (39%). In those cases where the most sensitive trophic group was not predicted correctly, the difference in measured values for the ECOSAR predicted and measured data trophic group ranged from just over 1 to 12000 (Figure 8).

There were 28 substances for which there were ECOSAR estimates and measured data for chronic toxicity to fish, daphnids and algae. ECOSAR predicted the most sensitive trophic group in nine of 28 cases (32%). In those cases where the most sensitive trophic group was not predicted correctly, the difference in measured values for the ECOSAR predicted and measured trophic group ranged from just over 1.5 to 657 (Figure 9).

These results suggest that for the types of substances for which EQS may be required, ECOSAR cannot be used to identify the most sensitive trophic group for empirical testing with an acceptable degree of reliability.

However, there are some occasions when there is a full measured acute base set of toxicity data for fish, invertebrates and algae, but one or more measured chronic data are missing. The effect of this is usually to increase the assessment factor from 10 to 50 (if one NOEC is missing), or from 10 to 100 (if two NOECs are missing) in line with guidance in the TGD (2003) and Lepper (2005). There were 26 substances in the dataset for which there were *both* acute and chronic measured data for fish, daphnids and algae. In 15 cases (58%) the most sensitive trophic group in the acute data set was also the most sensitive trophic group in the chronic data set. For the 11 substances where this was not the case, the difference in measured chronic values between the expected and observed most sensitive trophic group was less than ~5-fold for eight substances, and less than ~18-fold for a further two substances (Figure 10). The difference was larger for the endocrine active substance, bisphenol A, because algae were marginally more sensitive than fish and daphnids in the acute dataset, but the effects of bisphenol A on fish reproduction in chronic tests occurred at much lower levels than those affecting algal population growth. If these results are generally representative, it should be possible to use an ECOSAR estimate to fill a gap in a chronic dataset with a reasonable degree of confidence if the acute measured dataset does not identify the gap as potentially for the most sensitive trophic group. A precautionary assessment factor of 20 could be added to the PNEC derived from the most sensitive measured datum to account for the possibility that it is not from the most sensitive trophic group. However, this assessment factor would be lower than the factors of 50 and 100 that might currently be added if the TGD (2003) and Lepper (2005) approaches were followed.

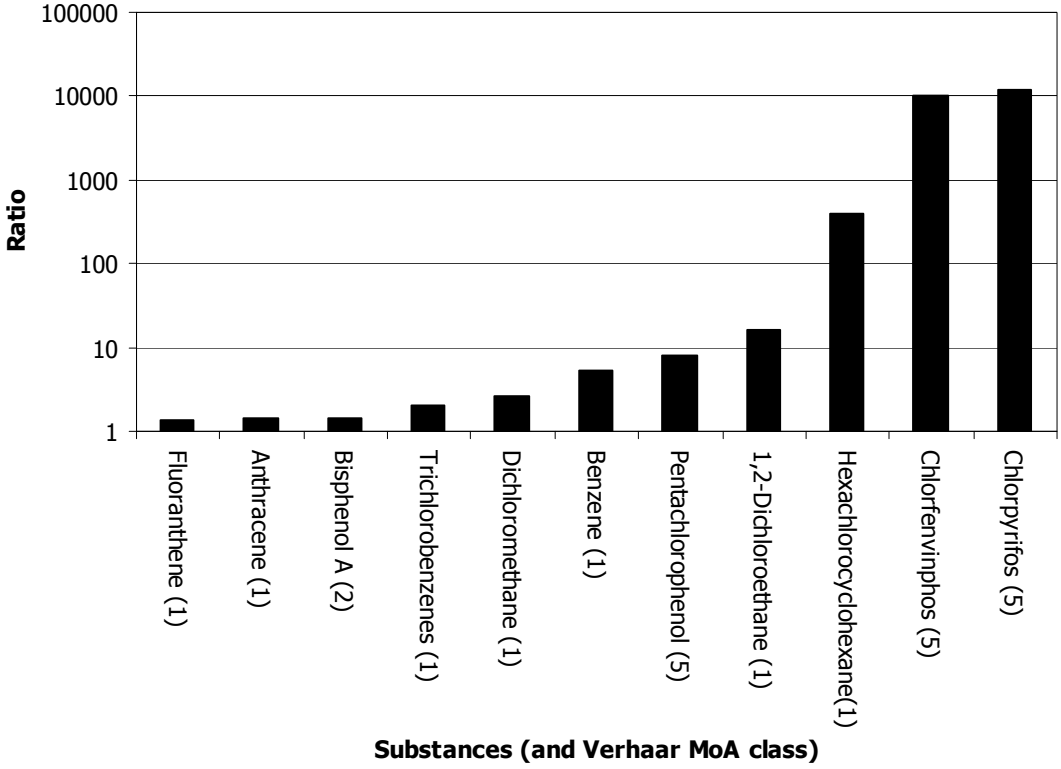



**Figure 8** **Ratio between measured values for most sensitive trophic group as identified by QSAR estimates and measured data (acute).**

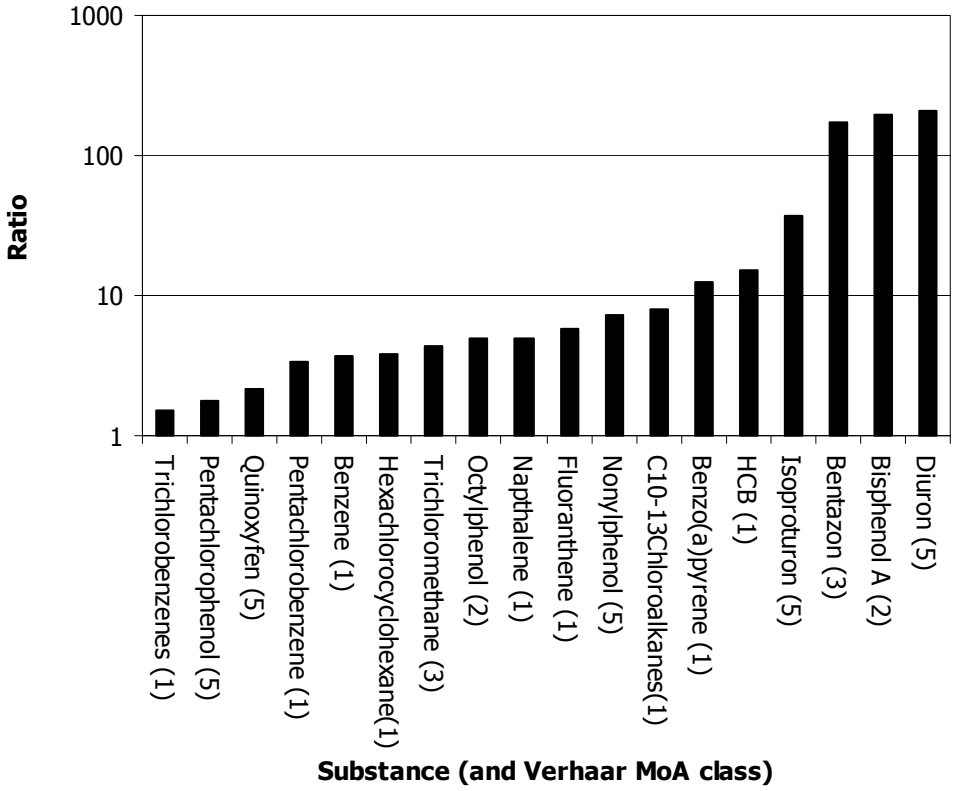



**Figure 9** **Ratio between measured values for most sensitive trophic group as identified by QSAR estimates and measured data (chronic).**

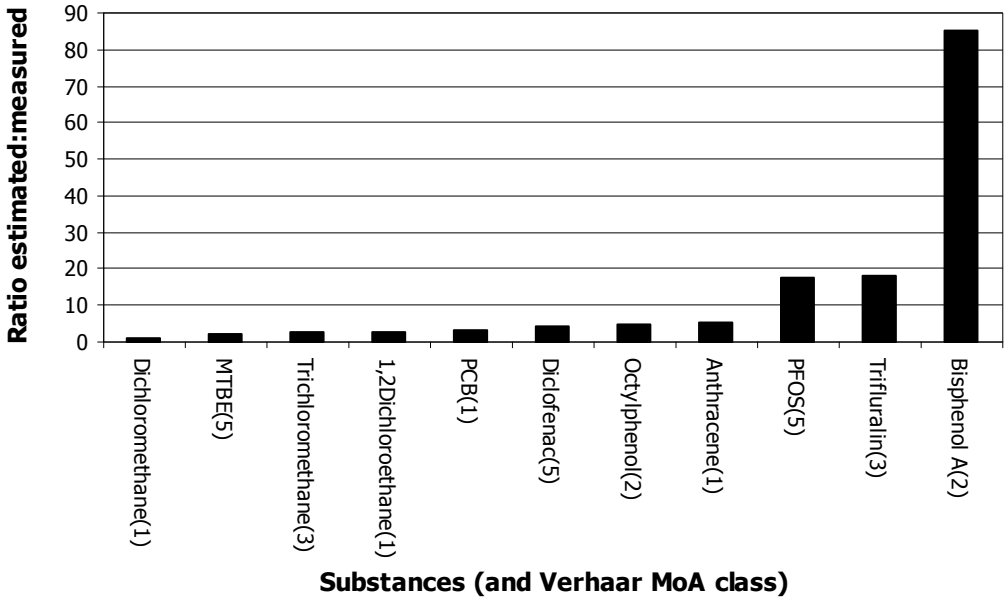



**Figure 10** **Ratio between measured chronic values for most sensitive trophic level and the trophic level expected to be most sensitive on the basis of acute data.**

## 3.6 Opportunities for read-across

Table 4 provides an example for PAHs of the category building approach, with substances ordered from lower to higher log Kow values. When data and estimates are ordered in this way a weight of evidence approach can be used to fill remaining gaps. For example, although there are no aquatic toxicity data for indeno (1,2,3-cd) pyrene, the physical properties of this substance plus QSAR estimates suggest that it should be no more toxic than benzo-g,h,i-perylene and no less toxic than benzo-k-fluoranthene. The measured data show that daphnids are consistently either as sensitive or more sensitive than fish or algae. They also show that, as found generally above, ECOSAR underestimated toxicity and did not identify daphnids as the most sensitive species. However, the weight of evidence suggests that daphnid data for benzo-g,h,i-perylene would be a reliable basis for read-across to an EQS for indeno(1,2,3-cd) pyrene without requiring use of any additional assessment factors.

The newly released OECD QSAR Application Toolbox (v1) makes the identification and building of defensible categories rapid, efficient and accurate. We used this tool to read-across to indeno(1,2,3-cd)pyrene as follows. Mechanistic profiling indicated no superfragments, no protein

binding, and failed to classify by either Cramer or Verhaar criteria. The program identified organic functional groups as arene and fused polycyclic aromatic, and DNA binding as polycyclic aromatic hydrocarbon. The EcoSAR classification was neutral organic, and OASIS acute toxicity MOA was basesurface narcotic. We defined a category on the basis of the EcoSAR classification and then gathered chronic fish, daphnid and algal data, as described in the methods section of this report.

The result of read-across to indeno(1,2,3-cd)pyrene for chronic fish toxicity (n=36 analogues) was a NOEC of 0.000112 mg $l^{-1}$ using the read-across function and 0.0013 mg $l^{-1}$ or 0.0002 mg $l^{-1}$ using, respectively, a linear ($r^2$=0.34) or quadratic ($r^2$=0.4) trend analysis function. This agrees well with the ecotoxicity trend data shown in table 4, indicating that indeno(1,2,3-cd)pyrene is between two to ten times more toxic to fish when compared with benzo-k-fluoranthene, if the trend analysis result is used. Gap filling by interpolation was possible for fish, making this estimate more reliable than gap filling by extrapolation, and allowing a high degree of confidence to be placed on estimated results.

The result of read-across for chronic daphnid toxicity (n=38 analogues) was a NOEC of 0.0467 mg $l^{-1}$ using the read-across function and 0.0023 mg $l^{-1}$ or 0.0000594 mg $l^{-1}$ using, respectively, a linear ($r^2$=0.61) or quadratic ($r^2$=0.65) trend analysis function. The result from linear extrapolation is about one order of magnitude higher than a value obtained by interpolation between values for benzo-a-pyrene and benzo-g,h,i-perylene in table 4, and the result from quadratic extrapolation is about the same as the value for benzo-g,h,i-perylene. This indicates that use of the value for the latter should be precautionary. Although gap filling was by extrapolation, the relatively good model fits as shown by the $r^2$ values, indicate that a reasonable degree of confidence can be placed on estimated results.

The result of read-across for chronic algal toxicity (n=11 analogues) was a NOEC of 1.24 mg $l^{-1}$ using the read-across function and 0.643 mg $l^{-1}$ or 0.00088 mg $l^{-1}$ using, respectively, a linear ($r^2$=0.0022) or quadratic ($r^2$=0.078) trend analysis function. The “analogues” for which suitable algal data were available turned out to have much lower logKow values than indeno(1,2,3-cd)pyrene, leading to a substantial degree of extrapolation rather than interpolation of toxicity data for gap filling. The linear extrapolation value contrasts markedly with measured values in Table 4, but the quadratic extrapolation produces a result very similar to measured values for benzo-a-pyrene and benzo-g,h,i-perylene. However, the very poor fit of both the linear and quadratic models as shown by the $r^2$ values suggests that only low confidence should be placed on estimated results.

**Table 4 Example of read-across matrix for PAHs proposed as WFD EQS.**

| Substance | Anthracene | Fluoranthene | Benzo-a-pyrene | Benzo-b-fluoranthene | Benzo-k-fluoranthene | Indeno(1,2,3-cd) pyrene | Benzo-g,h,i-perylene |
|---|---|---|---|---|---|---|---|
| **CAS RN** | 120-12-7 | 206-44-0 | 50-32-8 | 205-99-2 | 207-08-9 | 193-39-5 | 191-24-2 |
| **SMILES** | c(c(ccc1)cc(c2ccc3)c3)(c1)c2 | c(c(ccc1)ccc2)(c1c(c3ccc4)c4)c23 | c(c(c(cc1)ccc2)c2cc3)(c3cc(c4ccc5)c5)c14 | c12ccccc1cc3c4cccc4c5c3c2ccc5 | c2ccc1cc3c(cc1c2)c4cccc5cccc3c45 | c(c(c(c(ccc1)c2)c1cc3)c3cc4)(c2c(c5ccc6)c6)c45 | c16cccc2ccc3ccc4ccc5cccc6c5c4c3c12 |
| **Molecular weight** | 178.24 | 202.26 | 252.32 | 252.32 | 252.32 | 276.34 | 276.34 |
| **log Kow** | 4.35 (KOWIN); 3.45-4.54 (EC datasheet) | 4.93 (KOWIN); 5.13-5.33 (EC datasheet) | 6.11 (KOWIN); 5.97-6.15 (EC datasheet) | 6.11 (KOWIN); 6.04-6.57 (EC datasheet) | 6.11 (KOWIN); 6-6.84 (EC datasheet) | 6.7 (KOWIN); 4.19-6.4 (EC datasheet) | 6.7 (KOWIN); 6.18-7.23 (EC datasheet) |
| **Water solubility (mg/L)** | 3.177 (ECOSAR); 0.32-0.41 (EC datasheet) | 0.9232 (ECOSAR); 0.22-0.265 (EC datasheet) | 0.07207 (ECOSAR); 0.0034-0.0045 (EC datsheet) | 0.07207 (ECOSAR); No data (EC datasheet) | 0.07207 (ECOSAR); 0.00055-0.0011 (EC datasheet) | 0.01974 (ECOSAR); 0.00005-0.357 (EC datasheet) | 0.01974 (ECOSAR); 0.00016- 0.0003 (EC datasheet) |
| **Biodegradation (surface water DT50 (d))** | 60 (BIOWIN); 150 (EC datasheet) | 60 (BIOWIN); No information (EC datasheet) | 60 (BIOWIN); No information (EC datasheet) | 60 (BIOWIN); No information (EC datasheet) | 60 (BIOWIN); No information (EC datasheet) | 60 (BIOWIN); No information (EC datasheet) | 60 (BIOWIN); No information (EC datasheet) |
| **Bioaccumulation (fish)** | 530 (BCFWIN);162-9370 (EC datasheet) | 1900 (BCFWIN); 1700 (EC datasheet (calculated)) | 10000(BCFWIN); 146-2700 (EC datasheet) | 5600 (BCFWIN); No information (EC datasheet) | 10000 (BCFWIN); 8750 (EC datasheet (calculated)) | 25000 (BCFWIN); No information (EC datasheet) | 29000 (BCFWIN); 28183 (crustaceans, EC datasheet) |
| **Acute toxicity (mg/L) ECOSAR** | Fish (FW) 96-h LC50 = 0.817 | Fish (FW) 96-h LC50 = 0.264 | Fish and daphnid QSAR cutoff at log Kow=5 | | | Fish and daphnid QSAR cutoff at log Kow=5 | |
| | Fish (SW) 96-h LC50=0.583 | Fish (SW) 96-h LC50=0.249 | | | | | |
| | Daphnid 48-h LC50 =1.029 | Daphnid 48-h LC50 =0.346 | | | | | |

| Substance | Anthracene | Fluoranthene | Benzo-a-pyrene | Benzo-b-fluoranthene | Benzo-k-fluoranthene | Indeno(1,2,3-cd) pyrene | Benzo-g,h,i-perylene |
|---|---|---|---|---|---|---|---|
| | Mysid 96-h LC50=0.044 | Mysid 96-h LC50=0.009 | | | | | |
| | Algae 96-h EC50=0.737 | Algae 96-h EC50=0.256 | Algae 96-h EC50=0.029 | | | Algae QSAR cutoff at log Kow 6.4 | |
| **Acute toxicity (mg/L) Measured (EC datsheet)** | Fish (FW) 96-h LC50 = 0.0028-0.0265 (with UV exposure) | Fish (FW) 96-h LC50 = 0.036 | Fish (FW) 40.1-h LC50=0.0056 | ND | ND | ND | ND |
| | Daphnid 48-h LC50=0.036 | Daphnid 10.8-h LC50=0.009 | Daphnid 96-h LC50=0.005 | ND | ND | ND | ND |
| | Algae 96-h EC50=0.0039-0.0374 (with UV exposure) | Algae 7-d EC50=0.012 | Algae 96-h EC50=0.005 | ND | ND | ND | ND |
| **Chronic toxicity (mg/L) ECOSAR** | Fish 30-d ChV=0.154 | Fish 30-d ChV=0.055 | Fish 30-d ChV=0.006 | | | Fish 30-d ChV=0.002 | |
| | Daphnid 16-d EC50=0.148 | Daphnid 16-d EC50=0.064 | Daphnid 16-d EC50=0.011 | | | Daphnid 16-d EC50=0.005 | |
| | Algae 96-h ChV=0.286 | Algae 96-h ChV=0.139 | Algae 96-h ChV=0.032 | | | Algae 96-h ChV=0.017 | |
| **Chronic toxicity (mg/L) Measured (EC datasheet)** | Fish 4-d egg development NOEC=0.006 | Fish 41-d NOEC=0.0069 | Fish 42-d NOEC=0.0063 | ND | Fish 42-d NOEC=0.0027 | ND | ND |
| | Daphnid 21-d NOEC=0.0011 | Daphnid 7-d EC10=0.0012 | Daphnid 7-d EC10=0.0005 | ND | ND | ND | Daphnid 7-d EC10=0.00008 |
| | Algae 24-h NOEC=0.003 - 0.03 | Algae 7-d EC10=0.0016 | Algae 72-h EC10=0.00078 | ND | ND | ND | Algae NOEC=0.0012 |

# 4. Conclusions and Recommendations

The following conclusions and recommendations apply on the assumption that the substances analysed in this report are representative of the types of substances for which EQS may also need to be set in the future.

1. Identification of a substance as potentially PBT/vPvB is quite reliable using BIOWIN, BCFWIN and ECOSAR. However, reduction of the criteria thresholds for identifying a substance as *potentially* PBT when screened using only QSARs from those currently recommended by REACH would reduce the probability that substances fulfilling PBT criteria are missed. For example, in their QSAR-based PBT profiler (http://www.pbtprofiler.net/), USEPA considers a BCF>1000 as indicating bioaccumulation potential and toxicity of <0.1 mg $l^{-1}$ as indicating high concern.

2. The use of both measured and KOWWIN estimated log Kow values when estimating BCF with BCFWIN will also reduce the incidence of false negative results, as will the use of at least one other estimation program (e.g., VCCLAB or SPARC), although this will inevitably lead to an increase in false positive results at this screening stage, which is appropriate.

3. Mammalian toxicity QSARs are not sufficiently reliable at present for use in estimating secondary poisoning EQS. Therefore, if a substance is identified as bioaccumulative, measured toxicity values (e.g., on rats or mink) are currently necessary for assessment of secondary poisoning.

4. Sole reliance on QSARS in ECOSAR for estimating acute or chronic toxicity, and the subsequent use of these results for setting an EQS, is not recommended because of the tendency for ECOSAR to underestimate toxicity for the types of substances prioritised or proposed for EQS derivation, sometimes by a substantial amount.

5. Read-across using a weight of evidence approach and all relevant measured and estimated values for physical and ecotoxicological properties could be a valuable approach for deriving EQS if measured data are available for *interpolation* to the substance and endpoint(s) of interest, or if a reliable trend with low variability exists. The OECD QSAR Application Toolbox is a highly useful tool for read across, but should be used in a weight of evidence approach along with construction of a more traditional matrix of a few closely related analogues. The *de minimis* dataset for reliable read-across should consist of:
    a. Evidence of a consistent and reliable trend within a category of relevance to the endpoint of interest (e.g., a monotonic increase in log Kow with an increase in measured BCF and toxicity).
    b. Consistent and reliable measured values to identify the most sensitive trophic group, if toxicity is the endpoint of interest.
    c. Reliable measured data for the endpoint of interest that allow interpolation to a value for the substance of interest.
    d. QSAR estimates may be useful in a weight of evidence role for supporting read-across, but should not be used to replace the measured values identified in a – c above.

6. For a single substance for which reliable read-across is not possible the minimum data requirement for setting a freshwater chronic EQS, such as an annual average, should be a full freshwater acute toxicity base set, plus chronic data for the most sensitive trophic group in the

acute data set. If validated QSARs are available for this substance they can be used to estimate chronic toxic values for the two least acutely sensitive trophic groups. However, it would be prudent to apply an assessment factor >10 (probably 20) to the single measured chronic datum. This would account in most cases for any acute to chronic uncertainties in trophic level sensitivity.

7. Practical outstanding research needs of relevance to EQS derivation are:
    a. Further work on how to quantify substance persistence on the basis of QSAR biodegradation outputs such as BIOWIN.
    b. Comparison of bioaccumulation QSARs on substances likely to be prioritised for EQS derivation, in order to select a model that is more accurate than BCFWIN. The model proposed by Dimitrov et al. (2005) seems to show particular promise.

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European Commission

EUR 23758 EN – Joint Research Centre – Institute for Health and Consumer Protection

Title: Possible Application of Non-Testing Methods in Setting Environmental Quality Standards (EQS)

Author(s): Crane M, Watts C, Daginnus K and Worth A

Luxembourg: Office for Official Publications of the European Communities

2008 – 40 pp. – 21 x 29.7 cm
EUR – Scientific and Technical Research series – ISSN 1018-5593

Abstract

The Water Framework Directive is one of the most important pieces of European environmental legislation in recent years, requiring all inland and coastal waters to achieve ‘‘good status’’ by 2015. Article 16 of the Directive describes how and by when Environmental Quality Standards (EQS) for pollutants should be developed, and states that pollutants presenting a significant risk to or via water should be identified by the European Commission and classified as priority substances, with the most hazardous of these classified as priority hazardous substances.

Generation of ecotoxicity test data is one option for filling gaps when deriving EQS but there are also options that avoid testing, such as the use of (Quantitative) Structure Activity Relationships ([Q]SARs), Quantitative Structure-Property Relationships (QSPRs), Activity-Activity Relationships (AARs), Quantitative Structure Activity-Activity Relationships, or read-across from similar substances. All of these non-testing methods are based on the idea that properties (including biological activities) of a chemical substance depend on its intrinsic nature and can be directly predicted from its molecular structure and inferred from the properties of similar compounds whose activities are known.

This report explores application of the widely used and freely available EPIWIN suite of QSARs, particularly the ecotoxicity software ECOSAR, for derivation of EQS under the WFD. The predictive ability of these QSARs is examined using the 33 priority substances in the Water Framework Directive Daughter Directive on Priority Substances, plus the additional substances recommended for inclusion by the European Parliament, as representative substances for EQS derivation. We also investigated use of the OECD QSAR Application Toolbox version 1 for read across of data.

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